

sau96I
 avall
 asuI
 fnu4HI accI nlaIV
 286 GTA GCG GCG GAA GTC TAC GGG ACC GAA AGA CAG CCA CGA ACT CAC TAT
 78 Val Ala Ala Glu Val Tyr Gly Thr Glu Arg Gln Pro Arg Thr His Tyr

fnu4HI
 mboII bbvI aluI
 hphI fnu4HI aluI pvuII
 334 TAT GCC GTG GCT GTG GTG AAG AAG GGC GGC AGC TTT CAG CTG AAC GAA
 94 Tyr Ala Val Ala Val Val Lys Lys Gly Gly Ser Phe Gln Leu Asn Glu

haeIII sau96I
 stuI avall
 bglI haeI asuI foki
 382 CTG CAA GGT CTG AAG TCC TGC CAC ACA GGC CTT CGC AGG ACC GCT GGA
 110 Leu Gln Gly Leu Lys Ser Cys His Thr Gly Leu Arg Arg Thr Ala Gly

sau96I
 avall
 asuI
 nlaIV
 430 TGG AAT GTC CCT ACA GGG ACA CTT CGT CCA TTC TTG AAT TGG ACG GGT
 126 Trp Asn Val Pro Thr Gly Thr Leu Arg Pro Phe Leu Asn Trp Thr Gly

hgiIII aluI
 bsp1286 fnu4HI
 banII bbvI ddeI aluI
 ddeI mnlI pvuII mboII pvuII
 478 CCA CCT GAG CCC ATT GAG GCA GCT GTG CAG TTC TTC TCA GCC AGC TGT
 142 Pro Pro Glu Pro Ile Glu Ala Ala Val Gln Phe Phe Ser Ala Ser Cys

mspI
 hpaII
 scrFI
 nciI
 cauII
 526 GTT CCC GGT GCA GAT AAA GGA CAG TTC CCC AAC CTG TGT CGC CTG TGT
 158 Val Pro Gln Ala Asp Lys Gly Gln Phe Pro Asn Leu Cys Arg Leu Cys

nlaIV
 scrFI
 ecoRII
 mnlI bstNI rsaI
 574 GCG GGG ACA GGG GAA AAC AAA TGT GCC TTC TCC TCC CAG GAA CCG TAC
 174 Ala Gly Thr Gly Glu Asn Lys Cys Ala Phe Ser Ser Gln Glu Pro Tyr

FIG.-1

nlaIV
 hgiCI
 aluI banI ddeI bsmal bsmal
 622 TTC AGC TAC TCT GGT GCC TTC AAG TGT CTG AGA GAC GGG GCT GGA GAC
 190 Phe Ser Tyr Ser Gly Ala Phe Lys Cys Leu Arg Asp Gly Ala Gly Asp

 sau96I
 avall
 asuI
 ppuMI
 ecoD109I
 hgiAI
 bsp1286 mnlI mnlI
 670 GTG GCT TTT ATC AGA GAG AGC ACA GTG TTT GAG GAC CTG TCA GAC GAG
 206 Val Ala Phe Ile Arg Glu Ser Thr Val Phe Glu Asp Leu Ser Asp Glu

 718 GCT GAA AGG GAC GAG TAT GAG TTA CTC TGC CCA GAC AAC ACT CGG AAG
 222 Ala Glu Arg Asp Glu Tyr Glu Leu Leu Cys Pro Asp Asn Thr Arg Lys

 scrFI
 nciI
 mspI
 hpaII
 cauII
 xmaI sau96I
 smaI nlaIV
 scrFI
 nciI avall
 cauII
 avall asuI
 sau96I ppuMI
 haeIII nlaIV
 asuI ecoD109I nlaIII
 bsrI
 766 CCA GTG GAC AAG TTC AAA GAC TGC CAT CTG GCC CGG GTC CCT TCT CAT
 238 Pro Val Asp Lys Phe Lys Asp Cys His Leu Ala Arg Val Pro Ser His

 sfaNI
 fokI mnlI hinfI mboII
 bglI draIII
 814 GCC GTT GTG GCA CGA AGT GTG AAT GGC AAG GAG GAT GCC ATC TGG AAT
 254 Ala Val Val Ala Arg Ser Val Asn Gly Lys Glu Asp Ala Ile Trp Asn

 scrFI
 ecoRII
 bstNI hphI
 862 CTT CTC CGC CAG GCA CAG GAA AAG TTT GGA AAG GAC AAG TCA CCG AAA
 270 Leu Leu Arg Gln Ala Gln Glu Lys Phe Gly Lys Asp Lys Ser Pro Lys

FIG.-1

sau3AI
 mboI
 dpnI
 xhoII
 bstYI
 bglII
 aluI
 bstXI
 nlaIV
 910 TTC CAG CTC TTT GGC TCC CCT AGT GGG CAG AAA GAT CTG CTG TTC AAG
 286 Phe Gln Leu Phe Gly Ser Pro Ser Gly Gln Lys Asp Leu Leu Phe Lys

nlaIV
 hgiCI
 pleI
 hinfI
 mnlI bsp1286 mnlI
 taqI banI auaI hinfI
 958 GAC TCT GCC ATT GGG TTT TCG AGG GTG CCC CCG AGG ATA GAT TCT GGG
 302 Asp Ser Ala Ile Gly Phe Ser Arg Val Pro Pro Arg Ile Asp Ser Gly

mspI
 hpaII
 rsaI
 nlaIV
 fokI
 mnlI
 1006 CTG TAC CTT GGC TCC GGC TAC TTC ACT GCC ATC CAG AAC TTG AGG AAA
 318 Leu Tyr Leu Gly Ser Gly Tyr Phe Thr Ala Ile Gln Asn Leu Arg Lys

mspI
 hpaII
 scrFI
 nciI
 mnlI
 fnu4HI
 bbvI cauII
 hinfI
 hhaI
 1054 AGT GAG GAG GAA GTG GCT GCC CGG CGT GCG CGG GTC GTG TGG TGT GCG
 344 Ser Glu Glu Glu Val Ala Ala Arg Arg Ala Arg Val Val Trp Cys Ala

hinfI
 mstI
 fspI
 fnu4HI
 aluI hhaI
 bstXI
 alwNI bbvI
 bsrI
 1102 GTG GGC GAG CAG GAG CTG CGC AAG TGT AAC CAG TGG AGT GGC TTG AGC
 350 Val Gly Glu Gln Glu Leu Arg Lys Cys Asn Gln Trp Ser Gly Leu Ser

fnu4HI
 bbvI
 bspMI
 mnlI haeIII
 mnlI
 sfaNI
 1150 GAA GGC AGC GTG ACC TGC TCC TCG GCC TCC ACC ACA GAG GAC TGC ATC
 366 Glu Gly Ser Val Thr Cys Ser Ser Ala Ser Thr Thr Glu Asp Cys Ile

scrFI
 ecoRII
 bstNI
 bstXI
 aluI sfaNI nlaIII
 fokI mnlI
 1198 GCC CTG GTG CTG AAA GGA GAA GCT GAT GCC ATG AGT TTG GAT GGA GGA
 382 Ala Leu Val Leu Lys Gly Glu Ala Asp Ala Met Ser Leu Asp Gly Gly

FIG.-1

nlaIV
 hgiCI
 banI scrFI
 bI ecoRII

1678 GTT GCA TTT GTG AAA GAT GTC ACT GTC TTG CAG AAC ACT GAT GGA AAT
542 Val Ala Phe Val Lys Asp Val Thr Val Leu Gln Asn Thr Asp Gly Asn

				taqI										fnu4HI	
				mnlI								mnlI		bbvI	
		bglI								ddeI				atwI	
1774	CTG	TGC	CTC	GAT	GGC	AAA	CGG	AAG	CCT	GTG	ACT	GAG	GCT	AGA	AGC TGC
574	Leu	Cys	Leu	Asp	Gly	Lys	Arg	Lys	Pro	Val	Thr	Glu	Ala	Arg	Ser Cys

sau96I
 nlaIV
 nlaIII
 styI haeIII
 ncoI asuI hinfI nlaIII bsmalI fokI
 1822 CAT CTT GCC ATG GCC CCG AAT CAT GCC GTG GTG TCT CGG ATG GAT AAG
 590 His Leu Ala Met Ala Pro Asn His Ala Val Val Ser Arg Met Asp Lys

fnu4HI
ecoNI
alwNI
bbvI
1870 GTG GAA CGC CTG AAA CAG GTG CTG CTC CAC CAA CAG GCT AAA TTT GGG
606 Val Glu Arg Leu Lys Gln Val Leu Leu His Gln Gln Ala Lys Phe Gly

sau3AI
 mboI mspI
 dpnI hpaII
 xhoII scrFI
 bstYI nciI
 alwI cauII bsrI

1919 AGA AAT GGA TCT GAC TGC CCG GAC AAG TTT TGC TTA TTC CAG TCT GAA
 622 Arg Asn Gly Ser Asp Cys Pro Asp Lys Phe Cys Leu Phe Gln Ser Glu

[Page 5 of 6]

```

                                haeIII
                                haeI
                                eaeI          styl
                                ddeI          cfrI      pleI ncol
                                draIII       balI      hinfI
1966 ACC AAA AAC CTT CTG TTC AAT GAC AAC ACT GAG TGT CTG GCC AGA CTC
638 Thr Lys Asn Leu Leu Phe Asn Asp Asn Thr Glu Cys Leu Ala Arg Leu

                                sau96I
                                auaI
                                asuI
                                nlaIII      ndeI      sspI      nlaIV
2014 CAT GGC AAA ACA ACA TAT GAA AAA TAT TTG GGA CCA CAG TAT GTC GCA
654 His Gly Lys Thr Thr Tyr Glu Lys Tyr Leu Gly Pro Gln Tyr Val Ala

                                scrFI
                                ecoRII
                                bstNI
                                hgiAI
                                bsp1286      mnlI      mnlI
2062 GGC ATT ACT AAT CGT AAA AAG TGC TCA ACC TCC CCC CTC CTG GAA GCC
670 Gly Ile Thr Asn Leu Lys Lys Cys Ser Thr Ser Pro Leu Leu Glu Ala

                                ddeI
                                mstII
                                mnlI
                                eco8II
                                ecoRI      bsu36I
                                mbolI      sau96I      haeIII
                                mbolI      asuI      aluI
2110 TGT GAA TTC CTC AGG AAG TAA AACCGAAGAA GATGGCCCAG CTCCCCAAGA
685 Cys Glu Phe Leu Arg Lys DC*

                                styl
                                haeIII
                                sau96I
                                asuI
                                mbolI      scrFI
                                earI      ecoRII      nlaIV
                                ddeI      aluI      bstNI      ecoD109I nlaIV
2161 AAGCCTCAGC CATTCACTGC CCCAGCTCT TCTCCCAGG TGTGTTGGGG CCTTGGCTCC

                                ecoNI
                                fokI
                                ddeI
2221 CCTGCTGAAG GTGGGGATTG CCCATCCATC TGCTTACAAT TCCCTGCTGT CGTCTTAGCA

2281 AGAAGTAAAA TGAGAAATTT TGTTGATATT CAAAAAAAAA
>LENGTH: 2319

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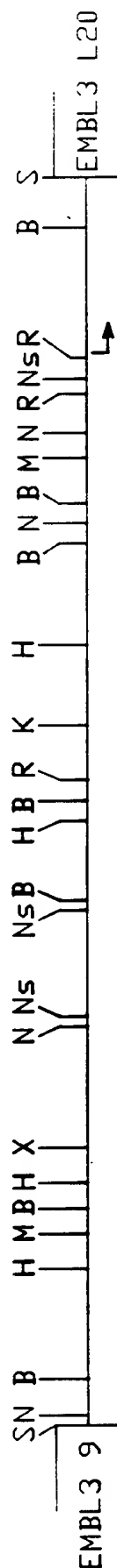
FIG.-1

1 GACTCCTAGG GGGTTGCAGA CCTAGTGGGA GAGAAAGAAC ATCGCAGCAG CCAGGACAGG TGAGGTGCAG GCTGGCTTTC CTCICGCAGC
 101 GCGGTGTGGA GTCTGTCTCT GCTCAGGGC TTTTCGAGC CTGGATCCTC AAGGAACAAG TAGACCTGGC CGCGGGGAGT GGGGAGGGAA GGGGTGTCTA
 201 TTGGGCAACA GGGCGGCAAA GCCCTGAATA AAGGGGCGCA GGGCAGGCGC AAGTGCAGAG CCTTCGTTTG CCAAGTCGCC TCCAGACCGC AGAC ATG AAA CTT
 -19 M K L
 304 GTC TTC CTC CTG CTG TTC CTC GGG GCC CTC GGA CTG TGT CTG GCT GGC CGT AGG AGA AGG AGT GTT CAG TGG TGC GCC GTA TCC
 -16 V F L L V L L F L G A L G L C L A G R R R S V Q W C A V S
 391 CAA CCC GAG GCC ACA AAA TGC TTC CAA TGG CAA AGG AAT ATG AGA AAA GTG CGT GGC CCT CCT GTG AGC TGC ATA AAG AGA GAC TCC
 14 Q P E A T K C F Q W Q R N M R K V R G P P V S C I K R D S
 478 CCC ATC CAG TGT ATC CAG GGC ATT GCG GAA AAC AGG GCC GAT GCT GTG ACC CTT GAT GGT GGT TTC ATA TAC GAG GCA GGC CTG GCC
 43 P I Q C I Q A I A E N R A D A V T L D G G F I Y E A G L A
 565 CCC TAC AAA CTG CGA CCT GTA GCG GCG GAA GTC TAC GGG ACC GAA AGA CAG CCA CGA ACT CAC TAT TAT GGC GTG GCT GTG GTG AAG
 72 P Y K L R P V A A E V Y G T E R Q P R T H Y Y A V A V A V V K
 652 AAG GGC AGC TTT CAG CTG AAC GAA CTG CAA GGT CTG AAG TCC TGC CAC ACA GGC CTT CGC AGG ACC GCT GGA TGG AAT GTC CCT
 101 K G G S F Q L N E L Q G L K S C H T G L R R T A G W N V P
 739 ACA GGG ACA CTT CGT CCA TTC TTG AAT TGG ACG GGT CCA CCT GAG CCC ATT GAG GCA GCT GTG GCC AGG TTC TCA GCC AGC TGT
 130 T G T L R P F L N V T G P E P I E A A V A R F F S A S C
 826 GTT CCC GGT GCA GAT AAA GGA CAG TTC CCC AAC CTG TGT CGC CTG TGT GCG GGG ACA GGG GAA AAC AAA TGT GGC TTC TCC TCC CAG
 159 V P G A D K G Q F P N L C R L C A G T G E N K C A F S S Q
 913 GAA CCG TAC TTC AGC TAC TCT GGT GCC TTC AAC GAT TGT CTG AGA GAC GGG GCT GGA GAC GTG GCT TTT ATC AGA GAG AGC ACA GTG TTT
 188 E P Y F S Y S G A F K C L R D G A G D V A F I R E S T V F
 1000 GAG GAC CTG TCA GAC GAG GCT GAA AGG GAC GAG TAT GAG TTA CTC TGC CCA GAC AAC ACT CGG AAG CCA GTG GAC AAG TTC AAA GAC
 217 E D L S D E A E R D E Y E L L C P D N T R K P V D K F K D
 1087 TGC CAT CTG GCC CGG GTC CCT TCT CAT GCC GTT GTG GCA CGA AGT GTG AAT GGC AAG GAG GAT GCC ATC TGG AAT CTT CTC CGC CAG
 246 C H L A R V P S H A V V A R S V N G K E D A I W N L L R Q
 1174 GCA CAG GAA AAG TTT GGA AAG GAC AAG TCA CCG AAA TTC CAG CTC TTT GGC TCC CCT AGT GGG CAG AAA GAT CTG CTG TTC AAG GAC
 275 A Q E K F G K D K S P K F Q L F G S P S G Q K D L L L F K D
 1261 TCT GCC ATT GGG TTT TCG AGG GTG CCC CCG AGG ATA GAT TCT GGG CTG TAC TCT ACT GGC TAC TTC ACT GGC ATC CAG AAC TTG
 304 S A I G F S R V P P R I D S G L Y L G S G Y F T A I Q N L
 1348 AGG AAA AGT GAG GAG GAA GTG GCT GCC CGG GTC GCG GTG GCG GAG CAG GAG CTG CGC AAG TGT AAC CAG
 333 R K S E E V A A R R A R V V C A V G E Q E L R K C N Q

FIG.-2

FIG.-2

1435 TGG AGT GGC TTG AGC GAA GGC AGC GTG ACC TGC TCC TCG GCC TCC ACC ACA GAG GAC TGC ATC GCC CTG GTG CTG AAA GGA GAA GCT
 362 V S G L S E G S V T C S S A S T T E D C I A L V L K G E A
 1522 GAT GCC ATG AGT TTG GAT GGA GGA TAT GTG TAC ACT GCA TGC AAA TGT GGT TTG GTG CCT GTC CTG GCA GAG AAC TAC AAA TCC CAA
 391 D A M S L D G G Y Y T A C K C G L V P V L A E N Y K S Q
 1609 CAA AGC AGT GAC CCT AAC TGT GTG GAT AGA CCT GTG GAA GGA TAT CTT GCT GTG GCG GTG GTT AGG AGA TCA GAC ACT AGC
 420 Q S S D P D P N C V D R P V E G Y L A V A V R R S D T S
 1696 CTT ACC TGG AAC TCT GTG AAA GGC AAG TCC TGC CAC ACC GCC GTG GAC AGG ACT GCA GGC TGG AAT ATC CCC ATG GGC CTG CTC
 449 L T W N S V K G K S C N T A V D R T A G V N I P M Q L L
 1783 TTC AAC CAG ACG GGC TCC TGC AAA TTT GAT GAA TAT TTC AGT CAA AGC TGT GCC CCT GGG TCT GAC CCG AGA TCT AAT CTC TGT GCT
 478 F N Q T G S C K F D E Y F S C A P G S D P R S N L C A
 1870 CTG TGT ATT GGC GAC GAG GGT GAG AAT AAG TGC CCC AAC AGC AAC GAG AGA TAC TAC GGC TAC ACT GGG GCT TTC CCG TGC
 507 L C I G D E Q G E N K C V P N S N E R Y Y G Y T G A F R C
 1957 CTG GCT GAG AAT GCT GGA GAC GTT GCA TTT GTG AAA GAT GTG ACT GTC TTG CAG AAC ACT GAT GGA AAT AAC AAT GAG GCA TGG GCT
 536 L A E N A G D V A F V K D V T V L Q N T D G N N E A W A
 2044 AAG GAT TTG AAC CTG GCA GAC TTT GCG CTG CTG TGC CTC GAT GGC AAA CGG AAG CCT GTG ACT GAC GCT AGA AGC TGC CAT CTT GCC
 565 K D L K L A D F A L L C L D G K R K P V T E A R S C H L A
 2131 ATG GCC CCG AAT CAT GCC GTG GTG TCT CCG ATG GAT AAG GTG GAA CGC CTG AAA CAG GTG CTC CAC CAA CAG GCT AAA TTT GGG
 594 M A P N H A V V S R M D K V E R L K Q V L L H Q Q A K F G
 2218 AGA AAT GGA TCT GAC TGC CCG GAC AAG TTT TGC TTA TTC CAG TCT GAA ACC AAA AAC CTT CTG TTC AAT GAC AAC ACT GAG TGT CTG
 623 R N G S D C P D K F C L F Q S E T K N L L F N D N T E C L
 2305 GCC AGA CTC CAT GGC AAA ACA TAT GAA AAA TAT TTG GGA CCA CAG TAT GTC GCA GGC ATT ACT AAT CTG AAA AAG TGC TCA ACC
 652 A R L H G K T T Y E K Y L G P Q Y V A G I T N L K K C S T
 2392 TCC CCC CTC CTG GAA GCC TGT GAA TTC CTC AGG AAG TAA AACGAAGAA GATGGCCAG CTCGCCAAGA AAGCTCAGC CATTCACTGC CCCCAGCTCT
 681 S P L L E A C E F L R K D
 2491 TCTCCCCAGG TGTGTTGGG CCTTGGCTCC CCTGCTGAAG GTGGGGATTG CCCATCCATC TGCTTACAAT TCCCTGCTGT CGTCTTAGCA AGAAGTAAAA
 2591 TGAGAAATTT TGTGATATT CAAAAAAA

RESTRICTION MAP OF 5' - FLANKING REGION OF α S1 CASEIN GENE

S - SalI

1Kb

B - BstII

R - EcoRI

H - HindIII

M - SmaI

N - NcoI

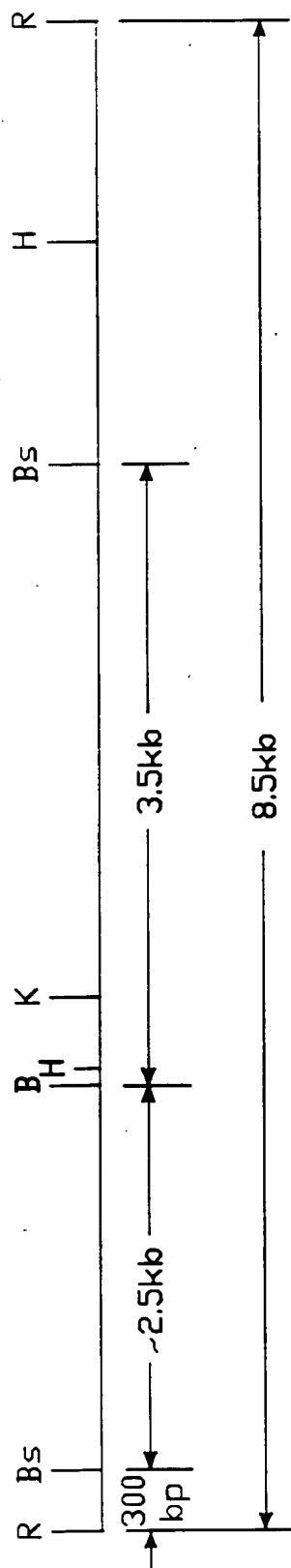
Ns - NsiI

X - XhoI

K - KpnI

FIG.-3

RESTRICTION MAP OF 3' FLANKING
REGION OF α S1 CASEIN GENE



1kb

R - EcoRI

Bs - Bst EII

B - BamHI

H - HindIII

K - KpnI

FIG.-4

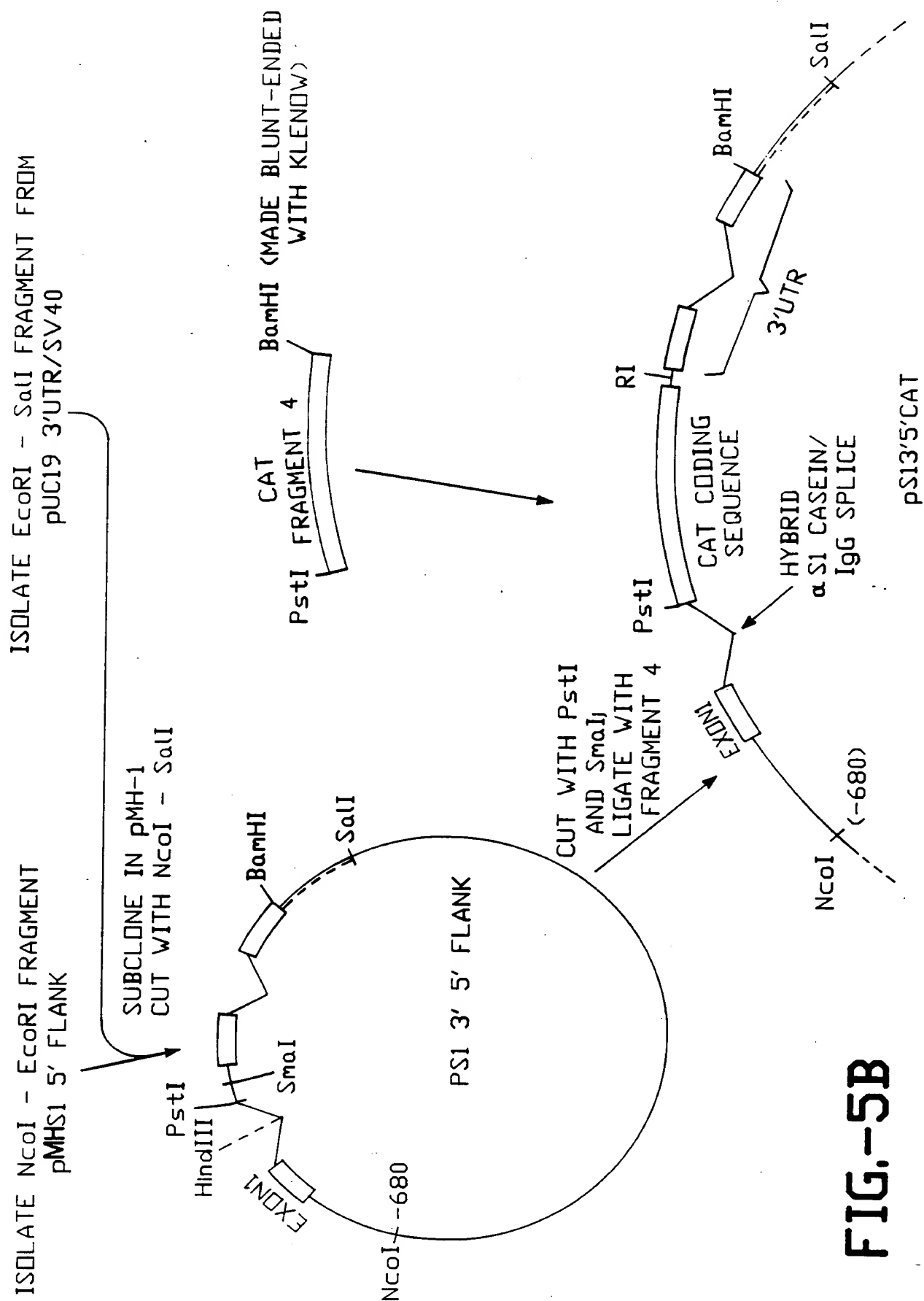


FIG.-5B

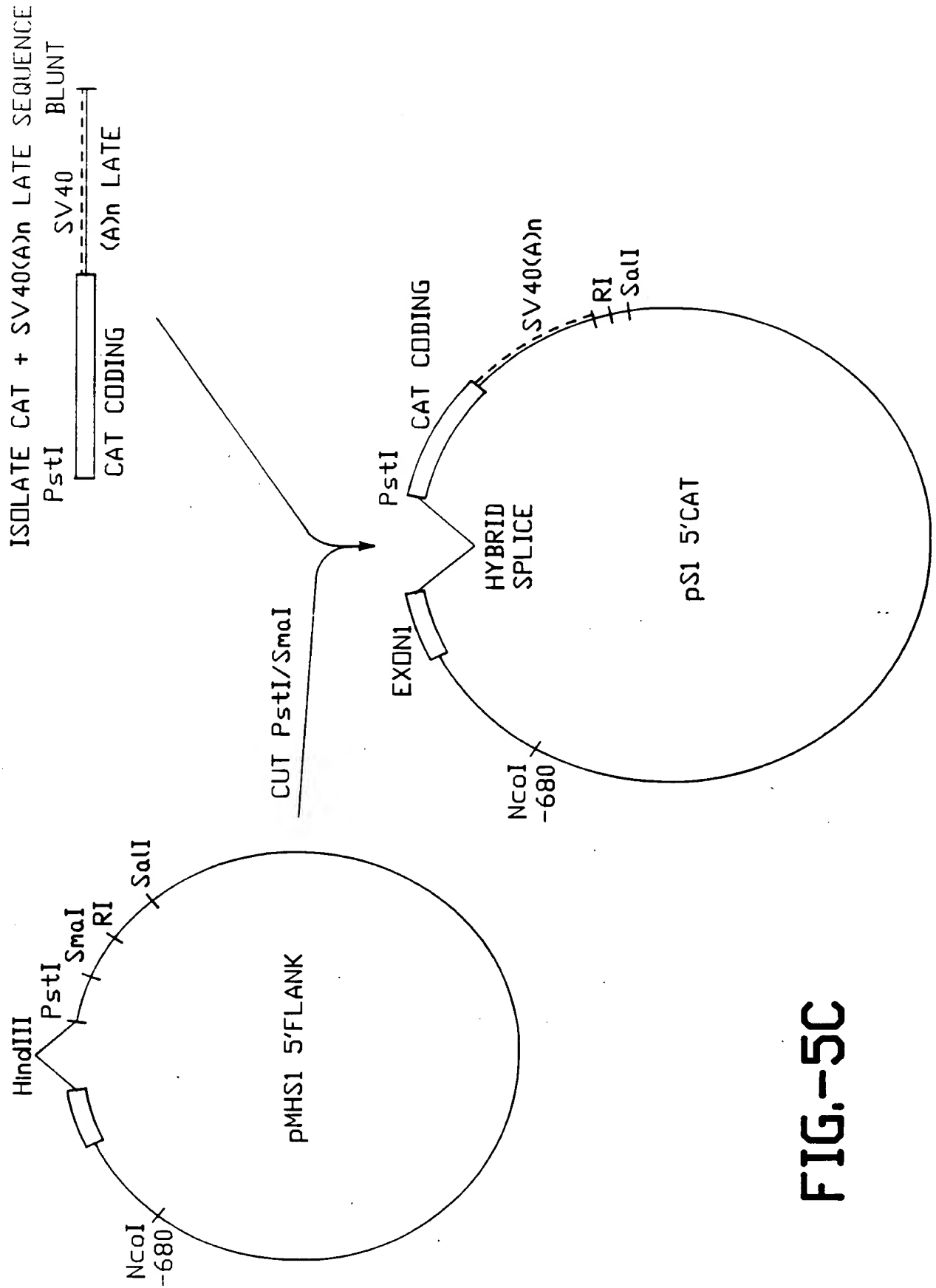


FIG.-5C

NAME pMH-1

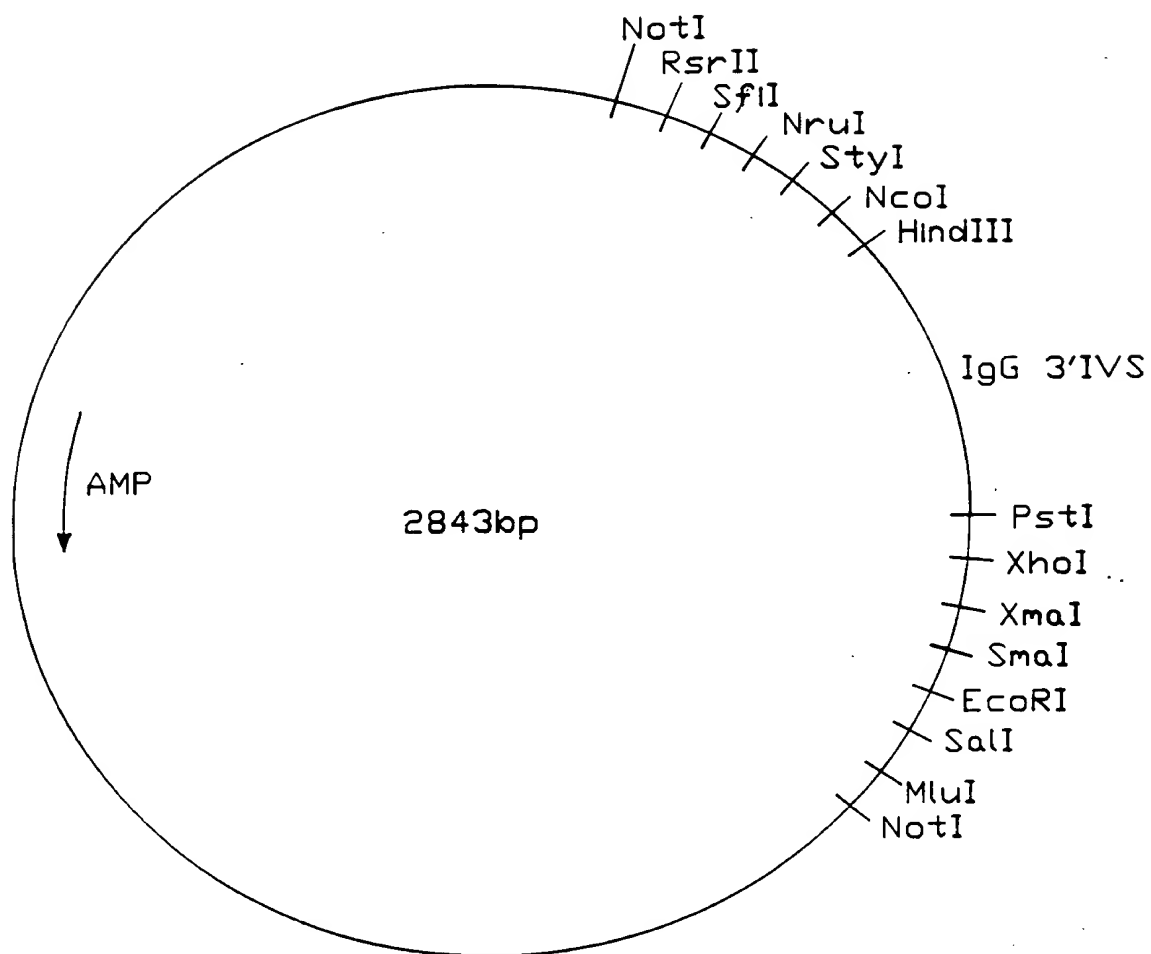
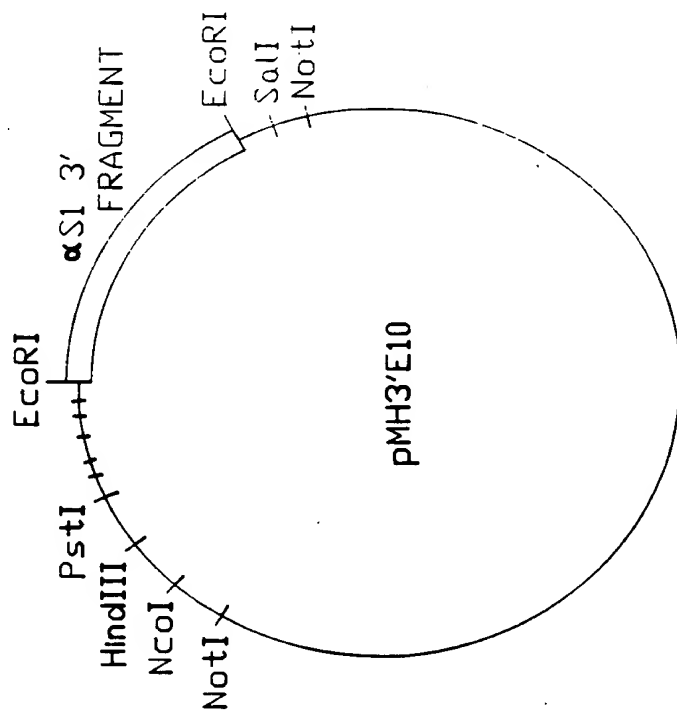


FIG.-6

- PLASMID **PMH3'E10** CONTAINS ~ 8.5Kb **EcoRI** FRAGMENT OF THE 3'-END OF α S1 CASEIN. PMH-1 WAS CUT W/**EcoRI** + LIGATED TO THIS FRAGMENT



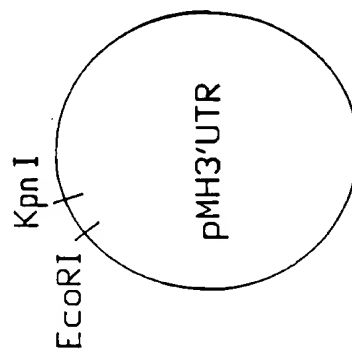
EcoRI

EcoRI

DIGEST

8.5 Kb α S1 CASEIN
+ 3' **EcoRI** FRAGMENT

- PLASMID **PMH3'UTRhLF2 LINKER**



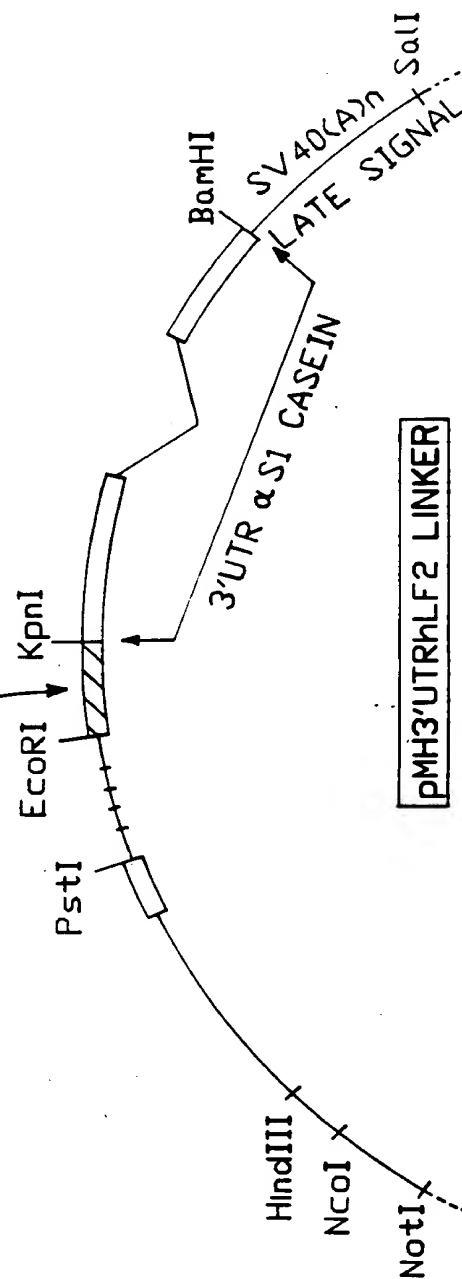
KpnI

EcoRI

PMH3'UTR

CUT W/**EcoRI** +
AND **KpnI**

(FRAGMENT 7)
3' TERMINAL
+ hLF CODING
SEQUENCE
(~ 20bp)



HindIII

NcoI

NotI

PstI

EcoRI

KpnI

BamHI

3'UTR α S1 CASEINSV40(A)_n

LATE SIGNAL

SalI

FIG.-7A

PMH3'UTRhLF2 LINKER

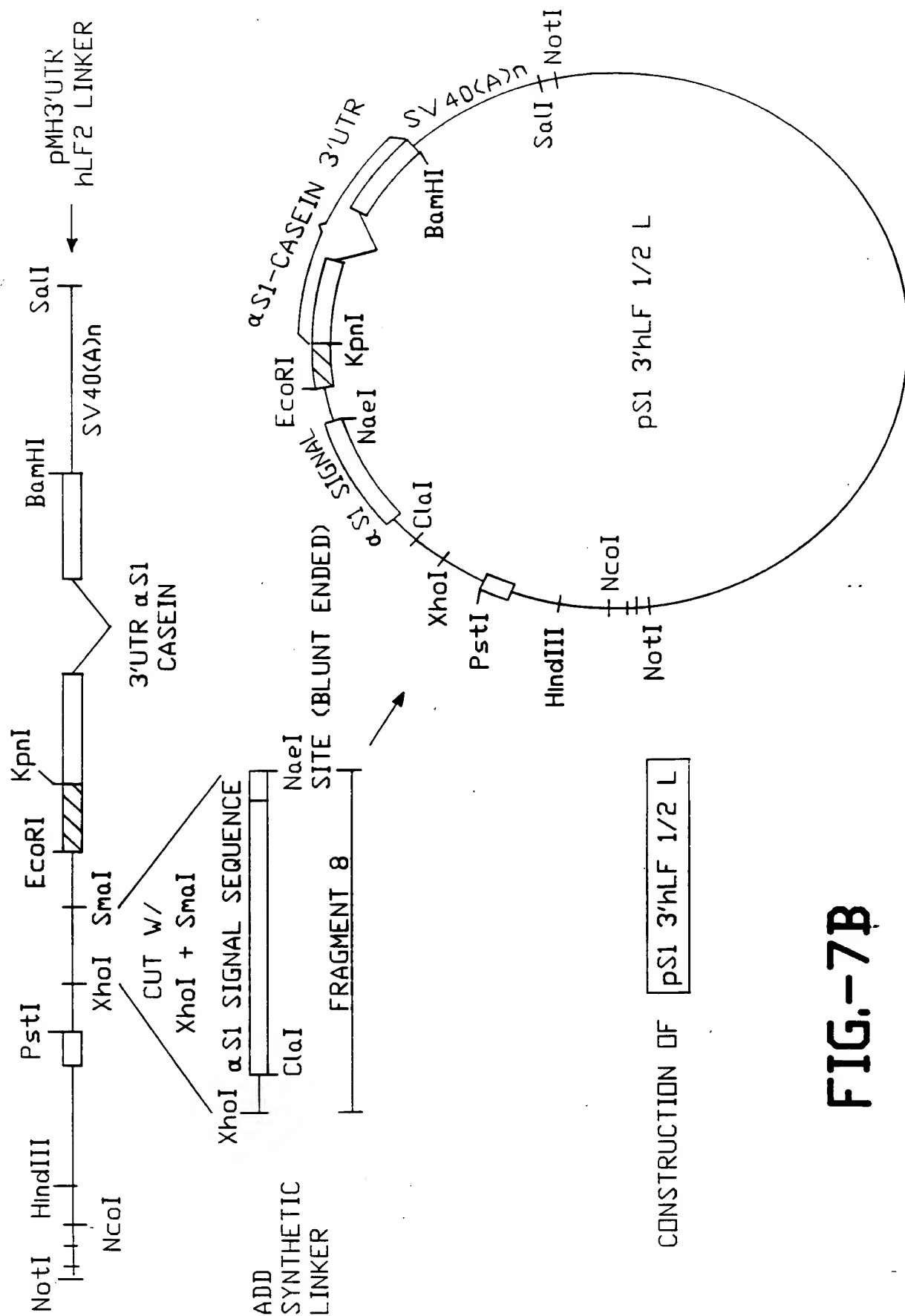


FIG.-7B

CONSTRUCTION OF pS1 3'UTRhLF

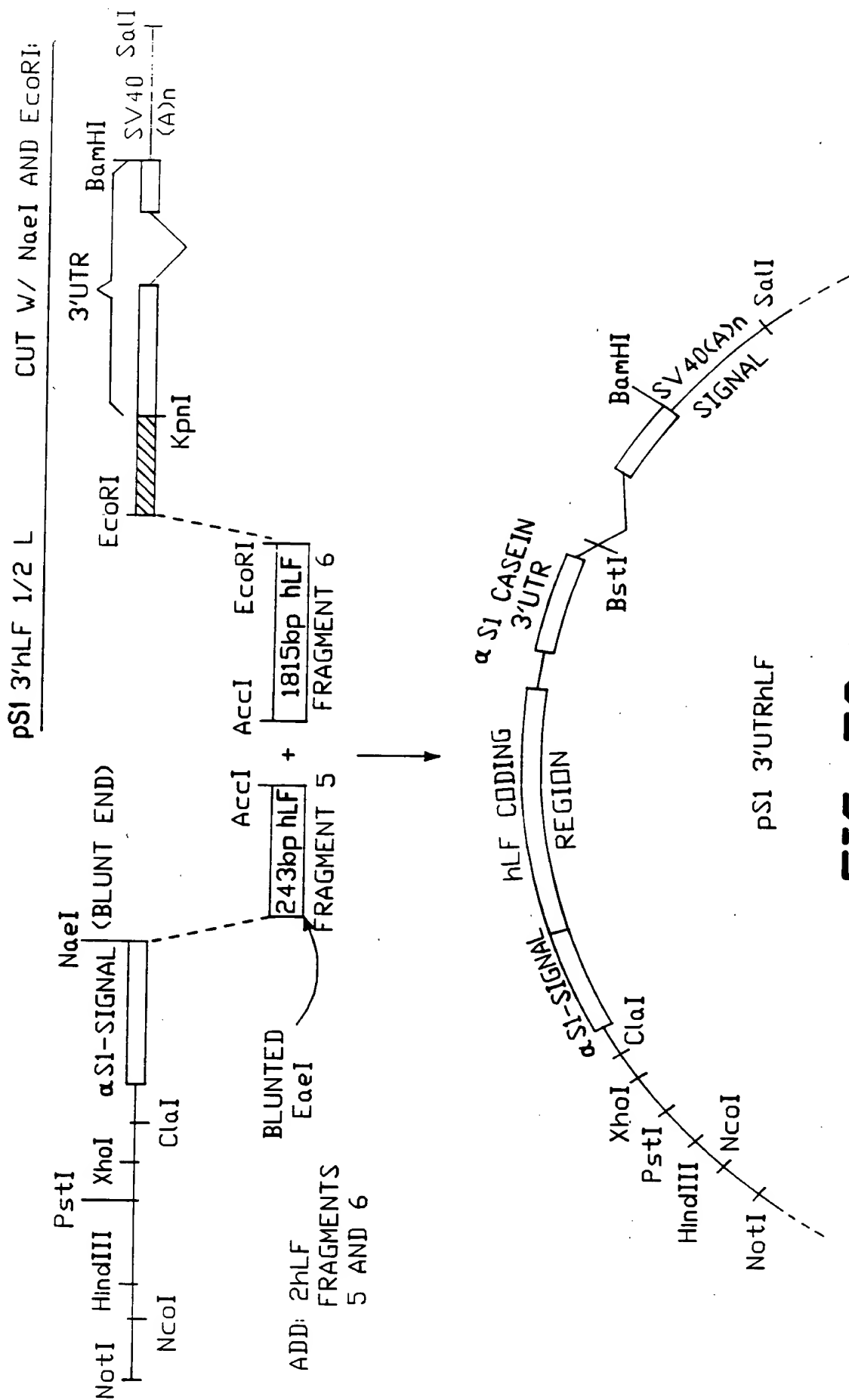


FIG.-7C

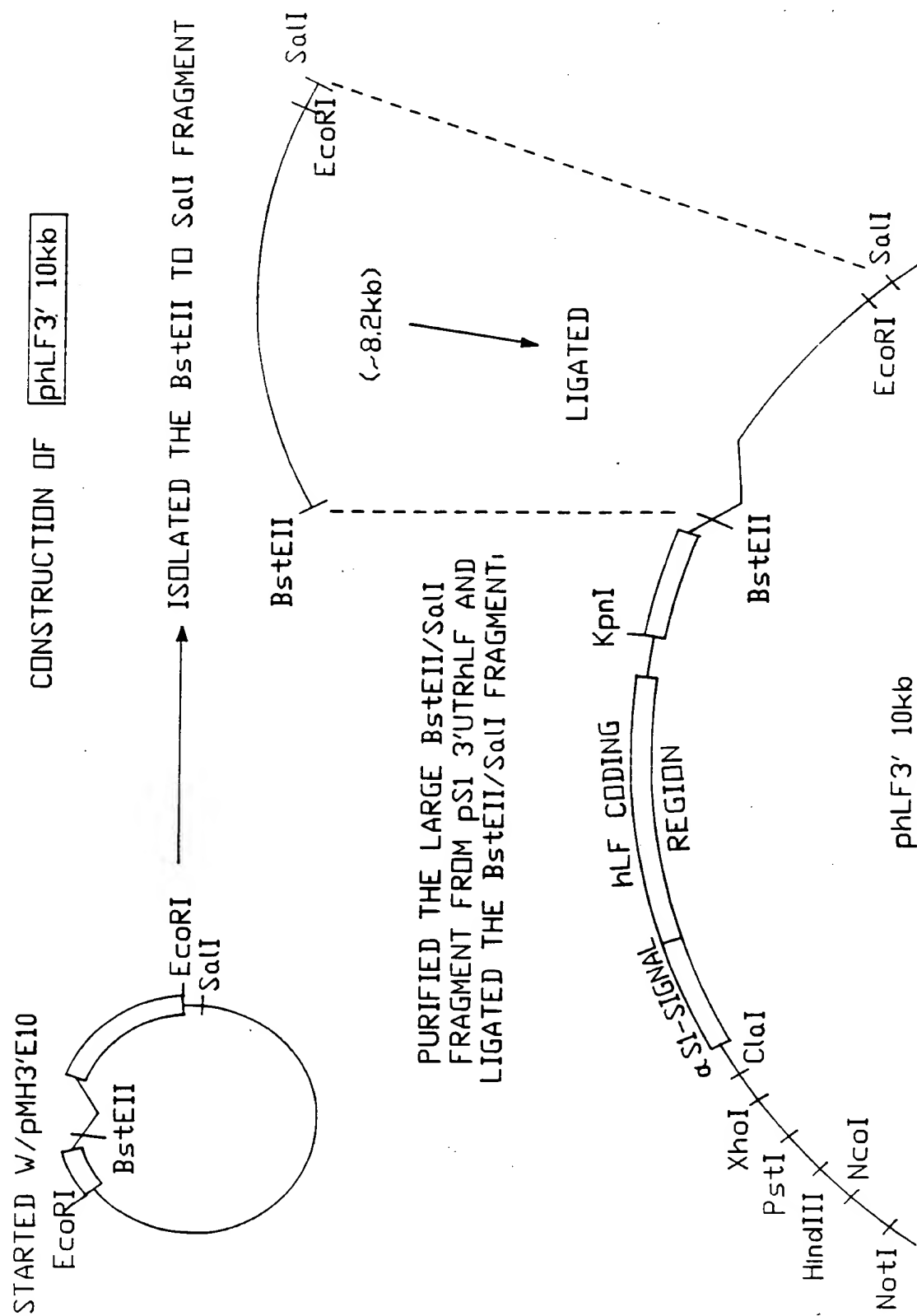
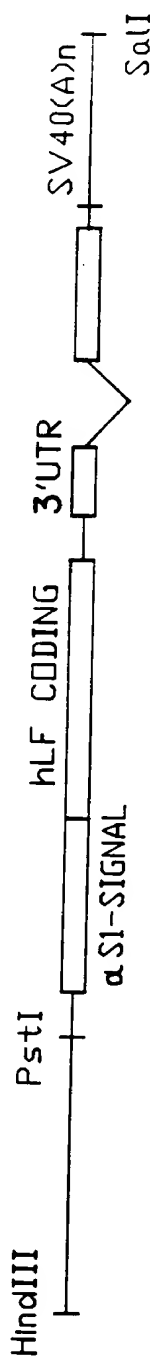


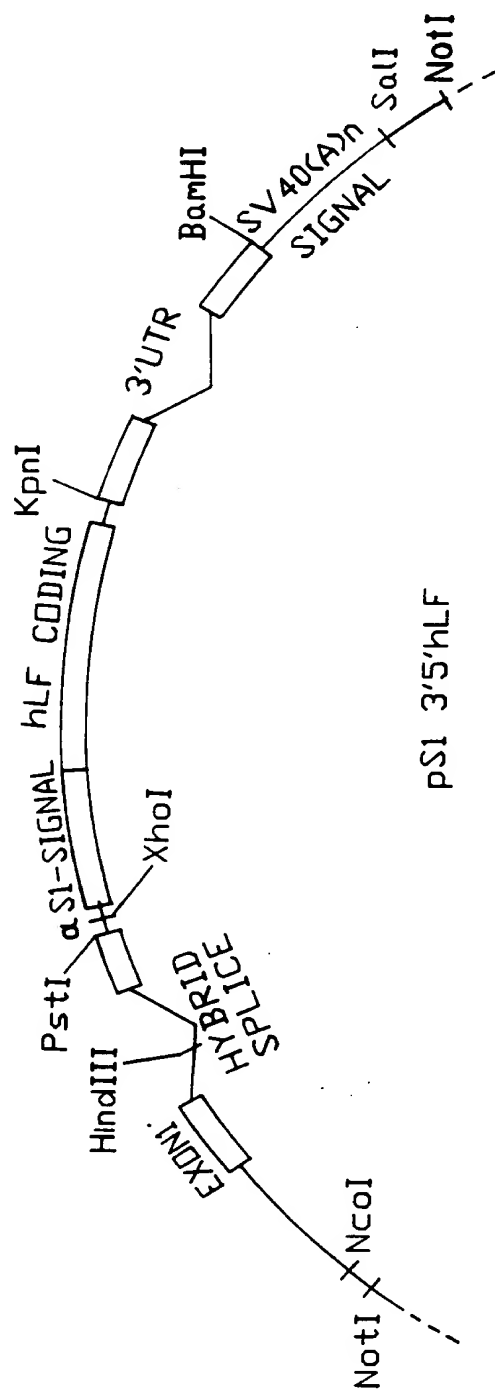
FIG.-7D

CONSTRUCTION OF **pS1 3'5'hLF**

STARTED W/pS1 3'UTRHLF. CUT W/HindIII AND SalI AND PURIFIED THIS FRAGMENT CONTAINING THE α S1-CASEIN SIGNAL SEQUENCE, hLF CODING REGION, α S1UTR AND SV40(A)_n.



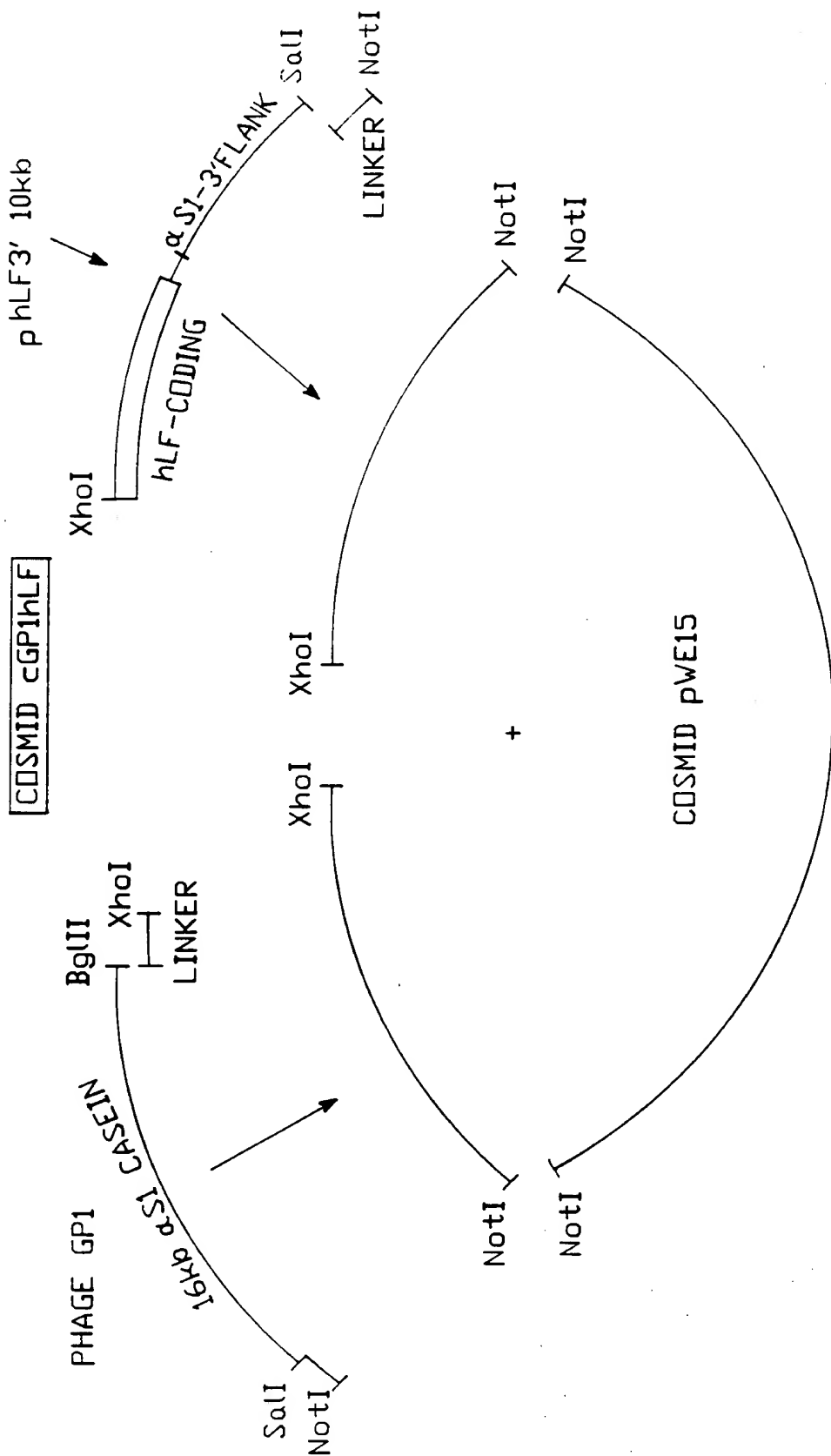
SUBCLONED INTO HindIII/SalI
CUT pMHS1 5' FLANK TO YIELD:



pS1 3'5'hLF

NOTE: **pS1 5'hLF** WAS MADE BY CUTTING **pS13'5'hLF** W/KpnI AND BamHI, FOLLOWED BY BLUNTING THE ENDS AND RELIGATING. THIS ELIMINATES THE SPLICED 3'UTR REGION.

FIG.-7E



3 WAY LIGATION. THE DNA FROM THIS COSMID IS PREPARED BY CUTTING WITH **NotI** AND PURIFYING THE EXPRESSION SEQUENCE PRIOR TO MICROINJECTION.

FIG.-7F

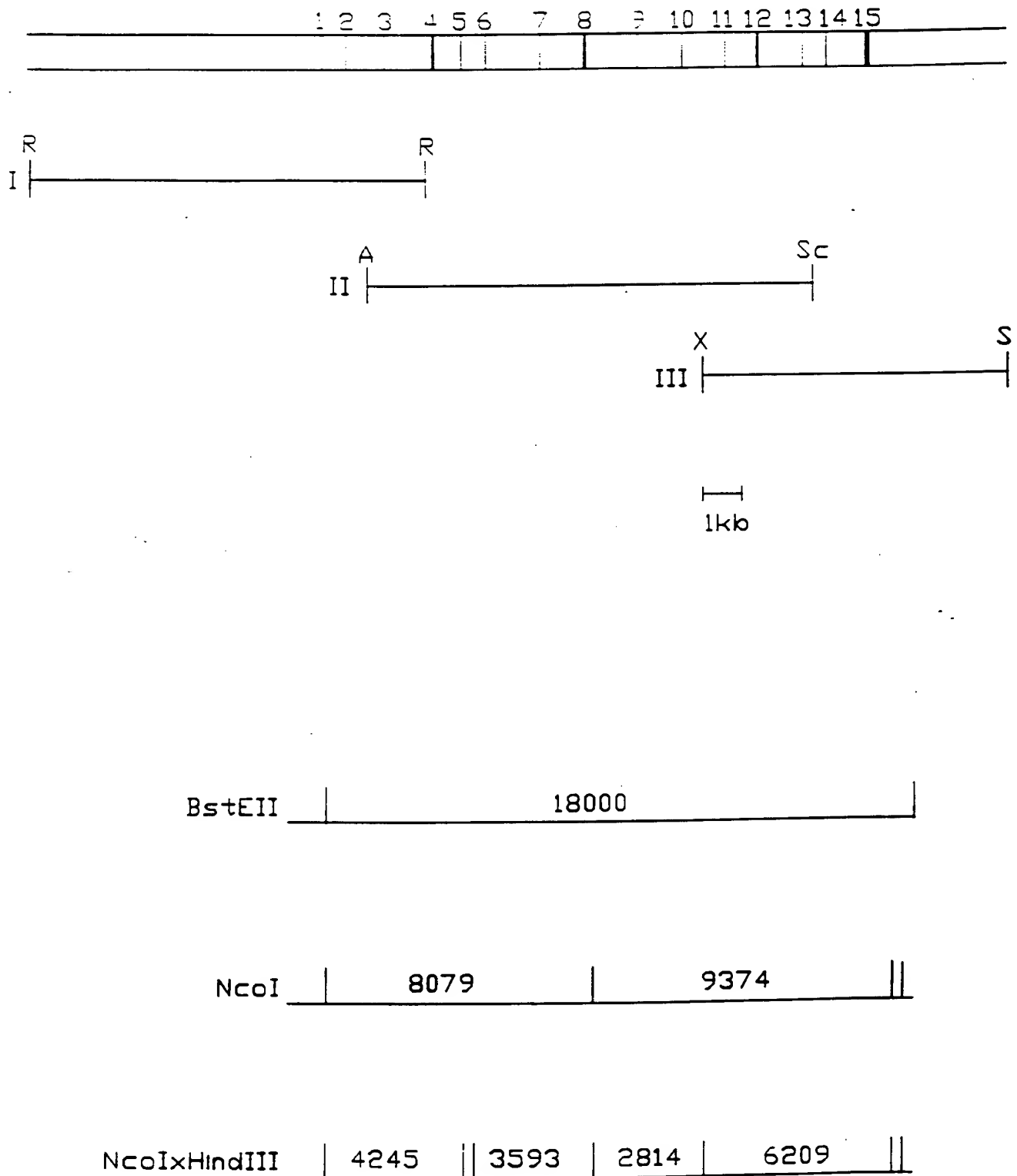
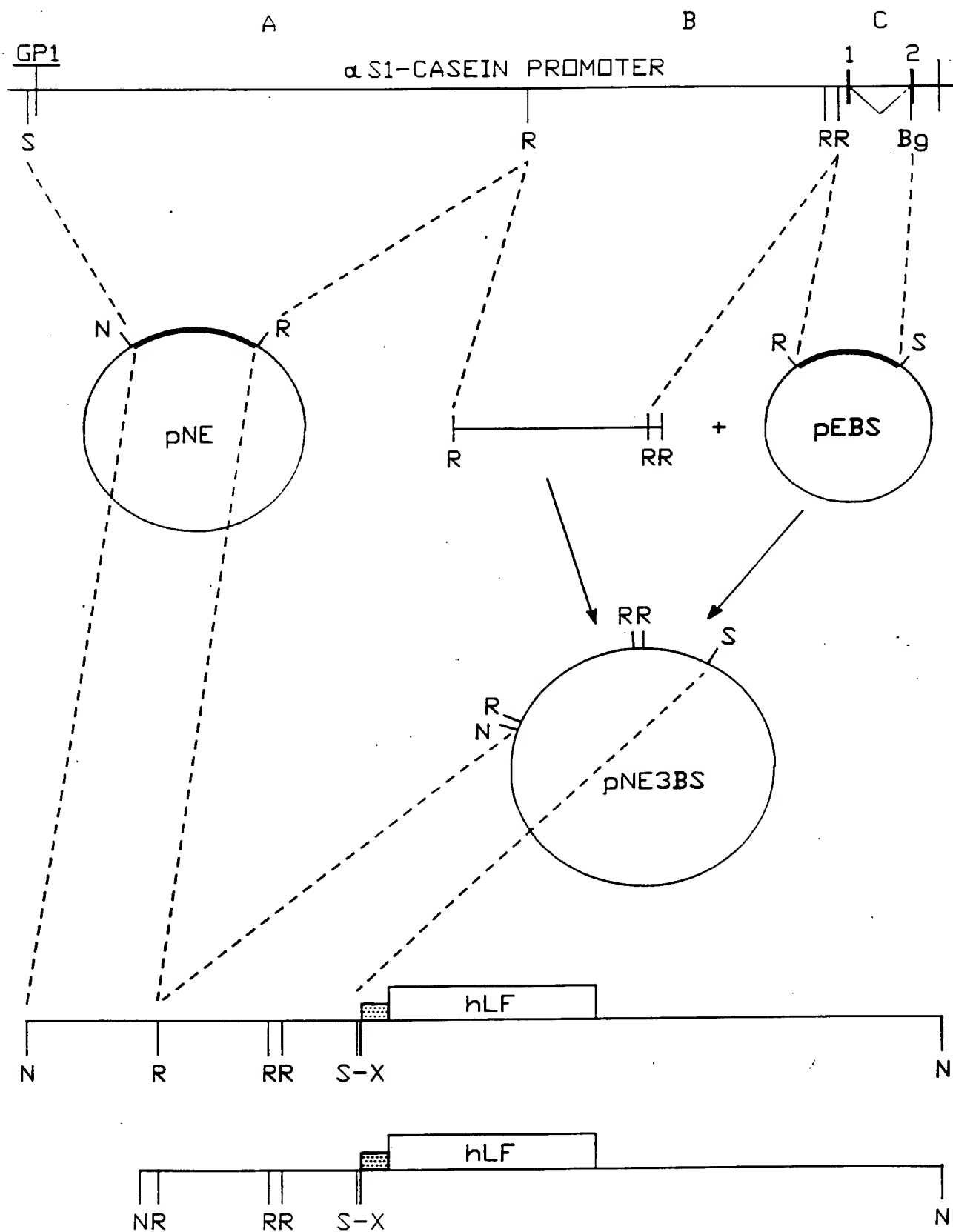


FIG.-8



S=SalI R=EcoRI Bg=BglII N=NotI X=XhoI

FIG.-9

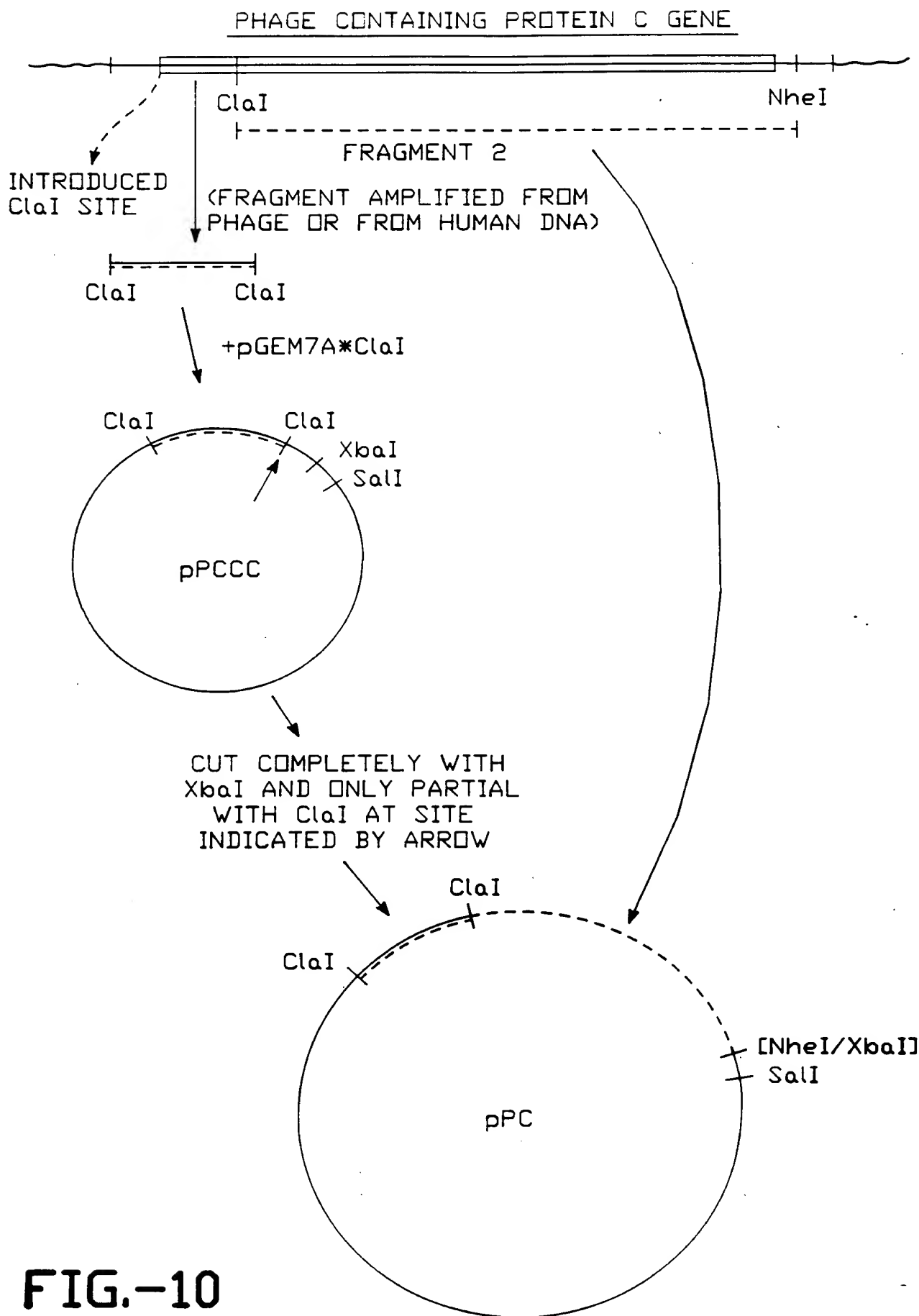


FIG.-10

5'- ATCACCTTGA TCATCAACCC AGCTTGCTGC TTCTTCCCAG
TCTTGGGTTC AAG gtattatgta tacatataac aaaatttcta tgattttcct ctgtctcacc ..
tttcattctt cactaatacg cagttgtaac ttttctatgt gattgcaagt attggtactt tcctatgata
tactgttagc aagcttgagg tgtggcaggc ttgagatctg gccatacact tgagtgacaa tgacatccac
tttgctttc tctccacag GTGTCCACTC CCAGGTCCAA CTGCAG -3'

FIG.-11

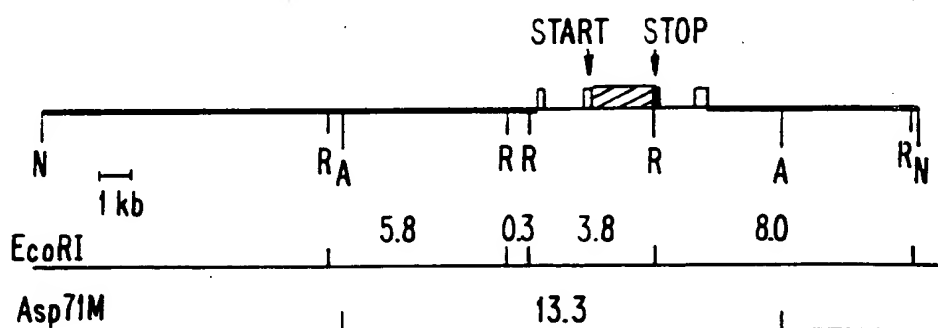


FIG. 12A.

COSMIDS CONTAINING ENTIRE hLF GENE (NOT DRAWN TO SCALE)

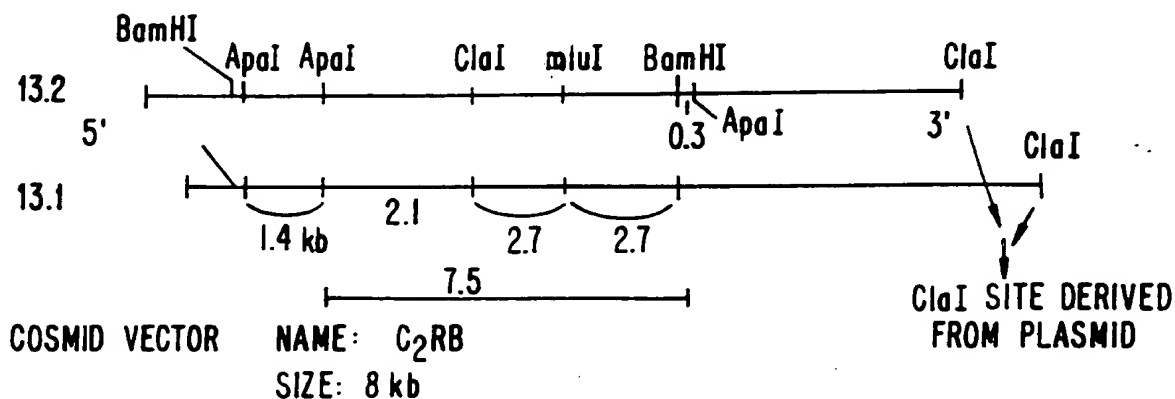


FIG. 13.

9 kb Bam HI hLF FRAGMENT IN pUC19

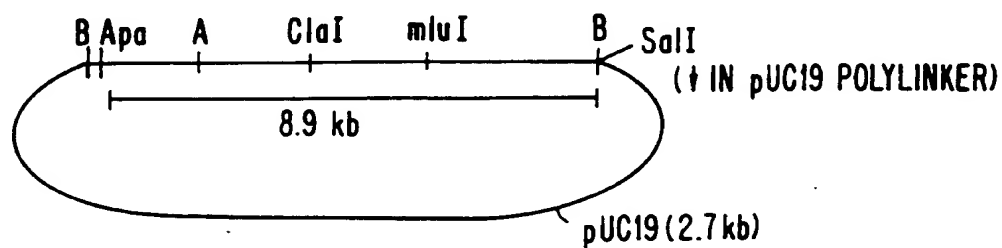
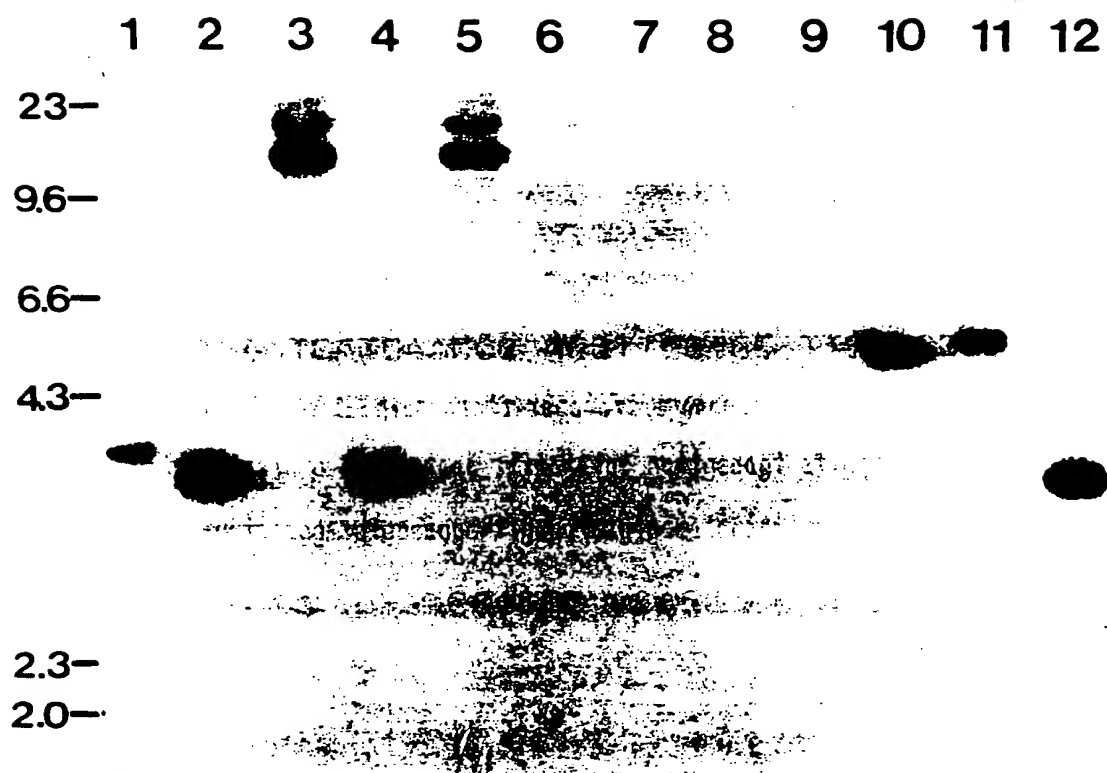
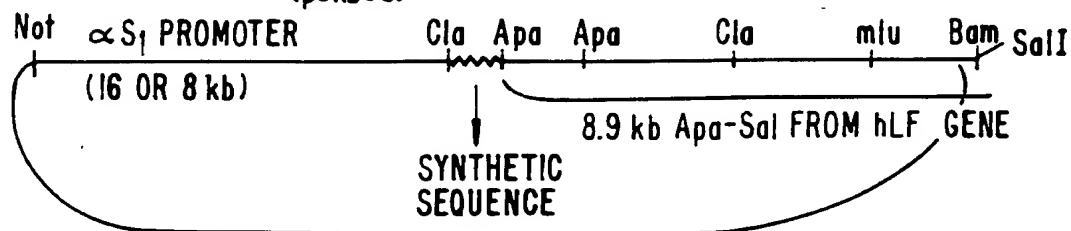


FIG. 14.

*FIG. 12B.*

LIGATION PRODUCT OF p16kbCS AND SYNTHETIC SEQ. (Cla-Apa)+ hLF FRAGMENT
(p8kbCS)



CLONING VECTOR: pkUN (4 kb)

CONSTRUCT NAME: 8 hLF gen 9k, OR 16 hLF gen.9k

FIG. 15A.

STRUCTURE OF ClaI-ApaI SYNTHETIC SEQUENCE

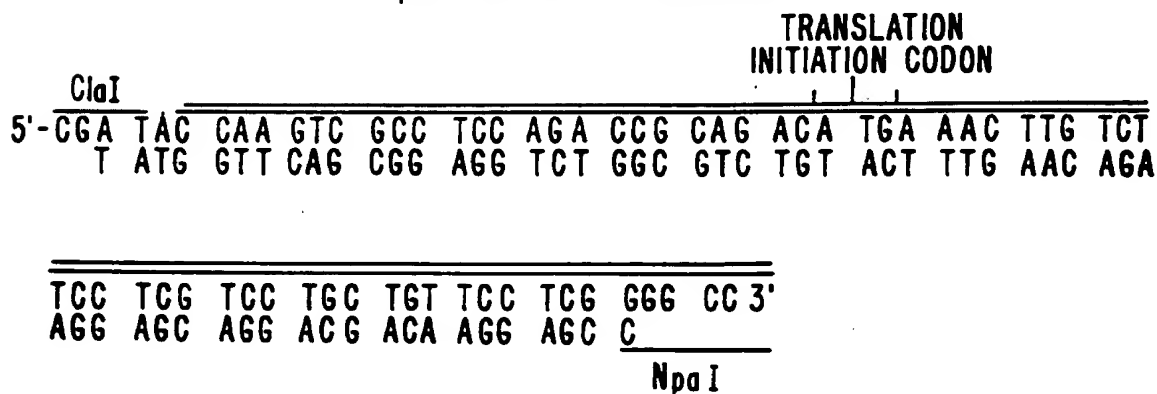
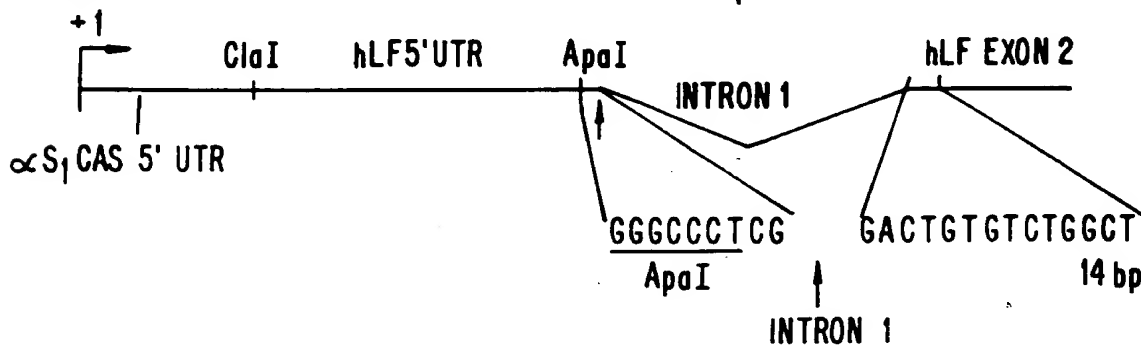


FIG. 15B.

+1: TRANSCRIPTION INITIATION SITE OF BOVINE αS_1 -CASEIN GENE



STRUCTURE OF REGION CONTAINING EXON 1 (HYBRID αS_1 -CASEIN/hLF EXON) AND PART OF EXON 2 OF THE GENOMIC hLF CONSTRUCTS DEPICTED IN FIGS. 15A THROUGH 17.

FIG. 15C.

COINJECTION

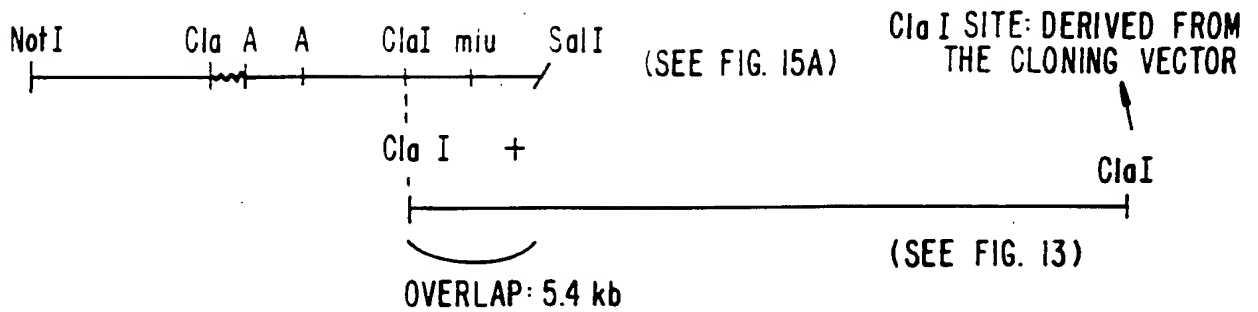
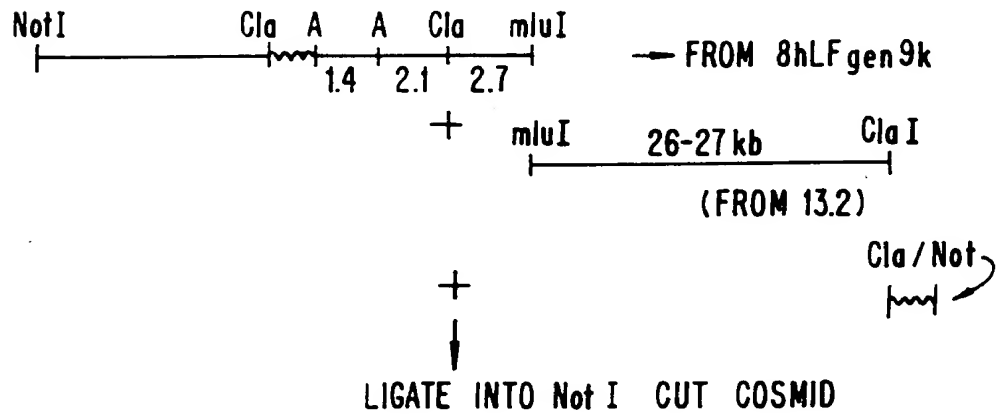


FIG. 16.

GENERATION OF 8hLF GENE



Cla/Not LINKER:

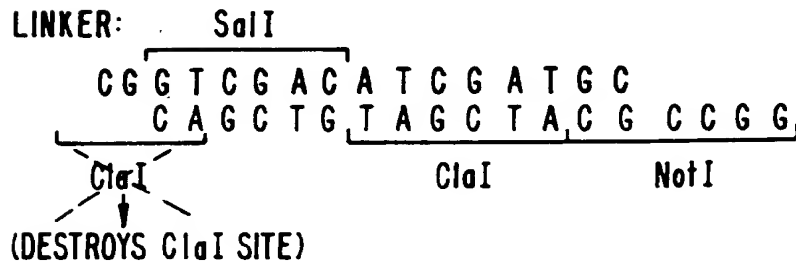


FIG. 17.

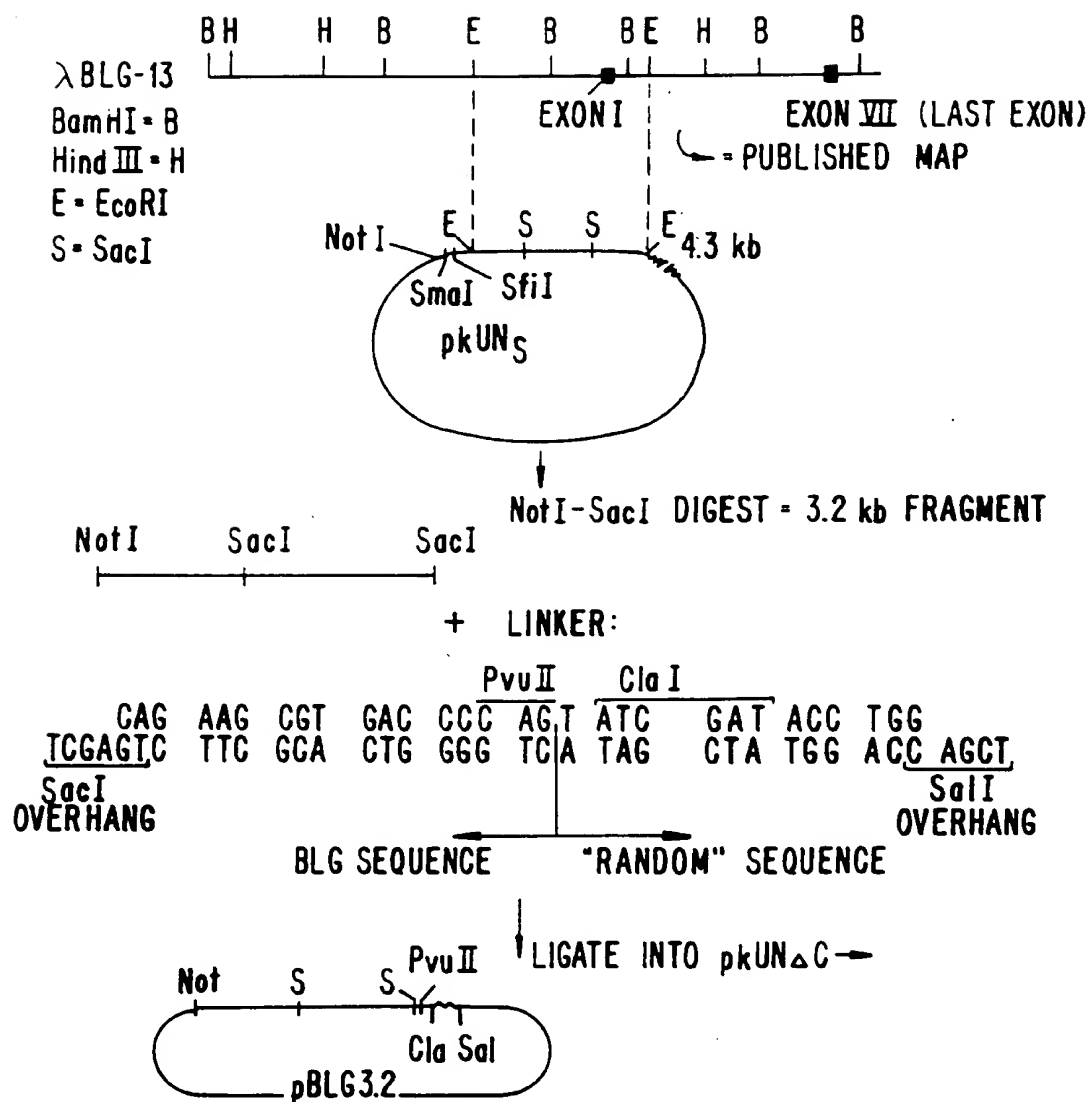


FIG. 18.

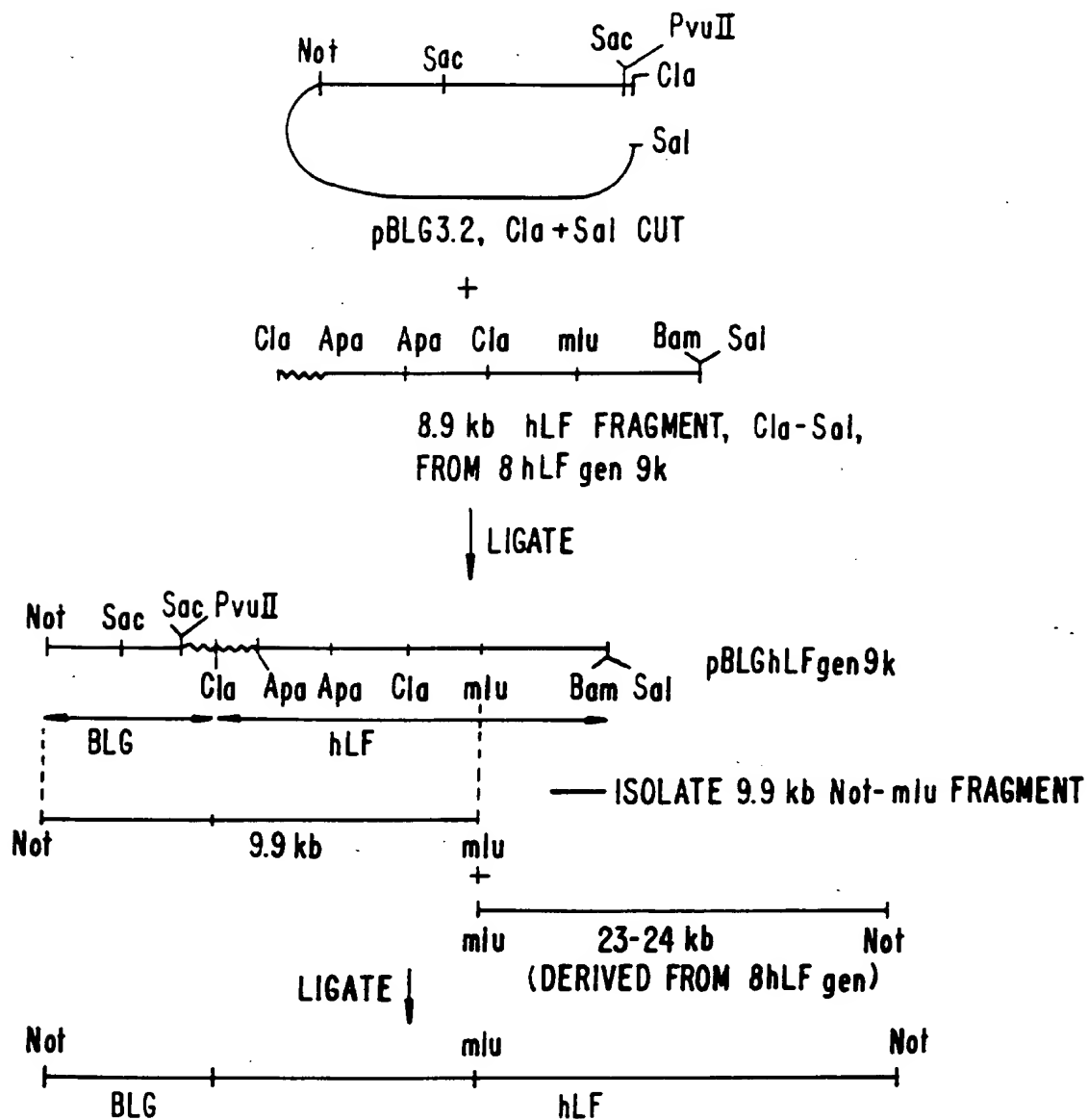


FIG. 19.

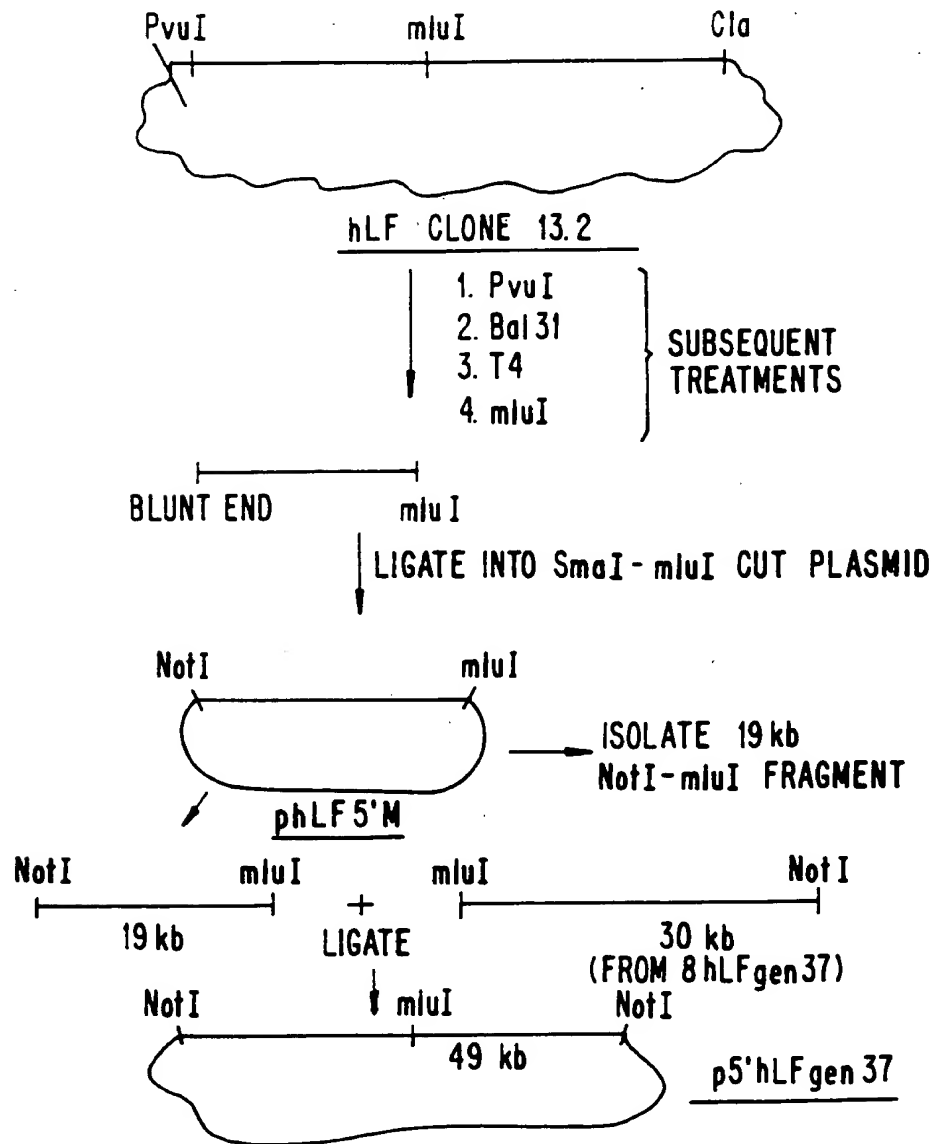


FIG. 20.

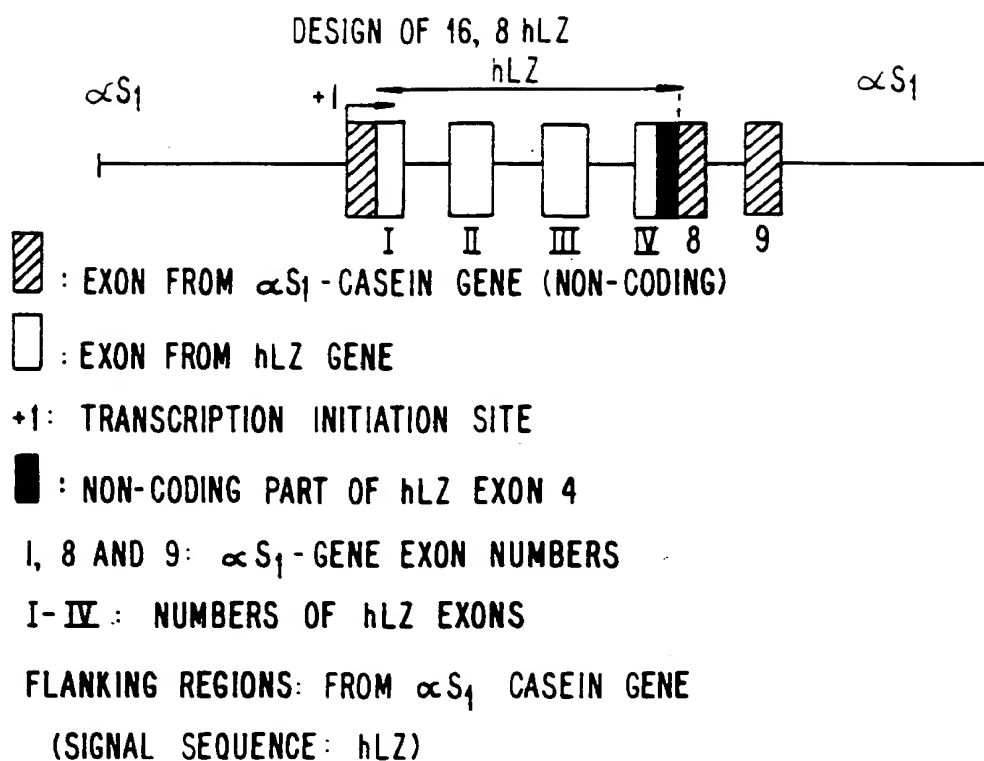


FIG. 21.

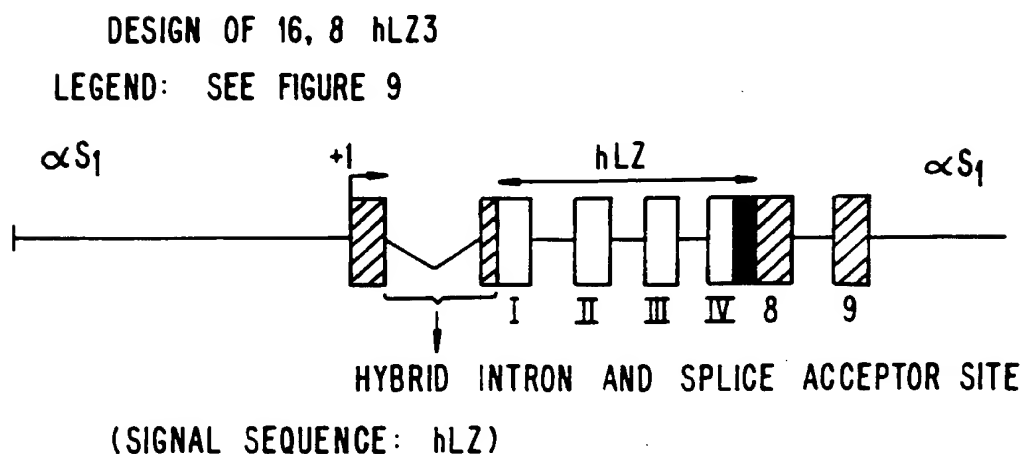
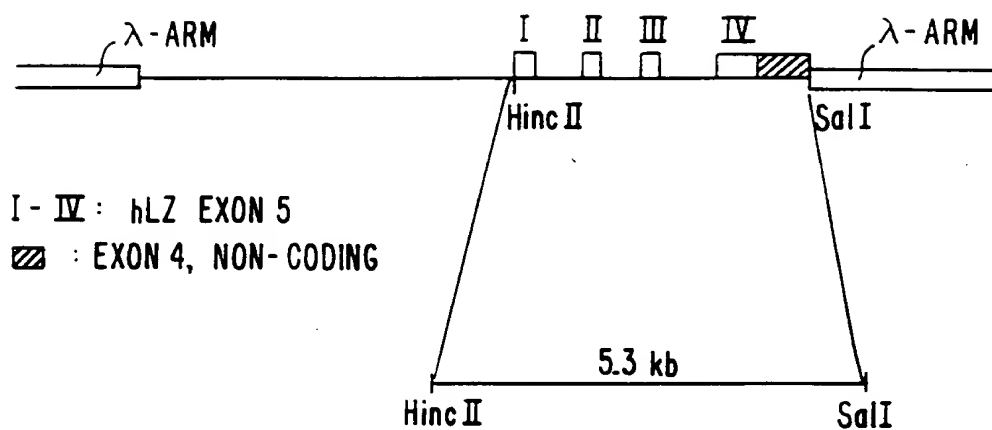
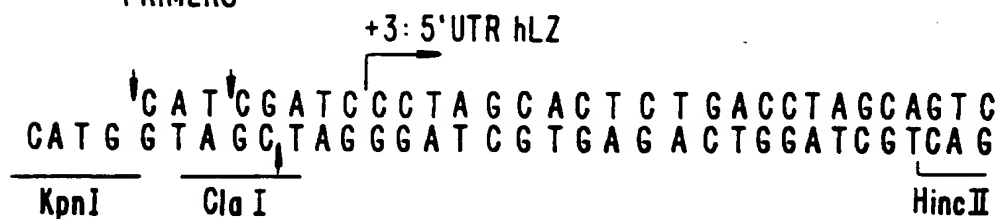


FIG. 22.

λ 7.2.1



PRIMERS:



LIGATE 5.3 Hinc II - Sal I
 Kpn - Hinc II

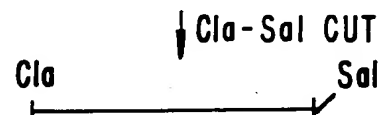
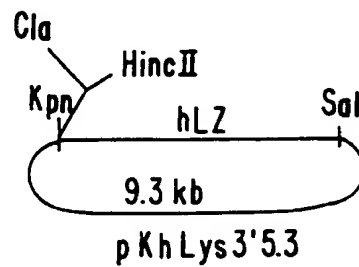


FIG. 23A.

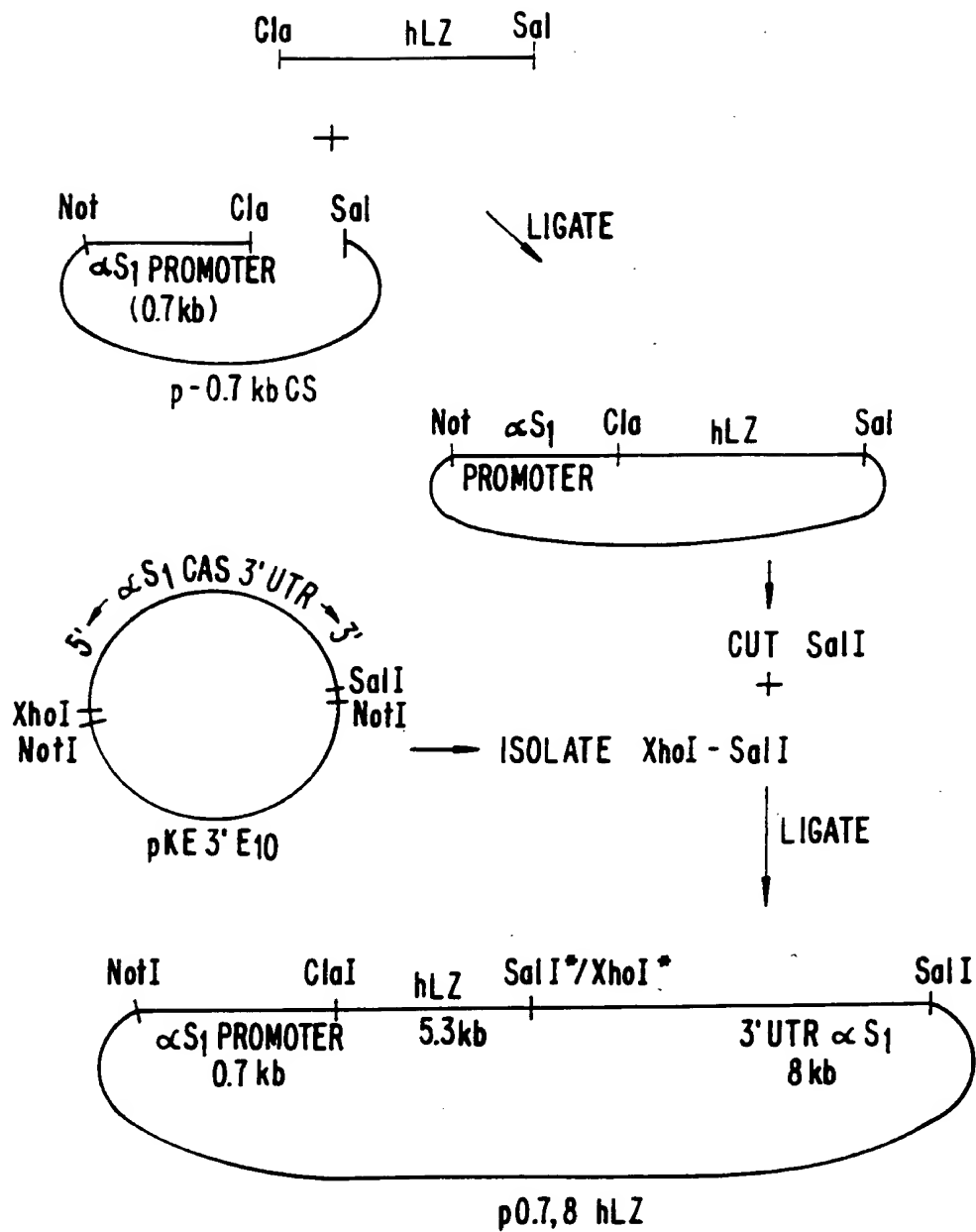


FIG. 23B.

LINKER S₁/S₂:SalI[⊗] - NotI - SalI[⊗]

⊗: = DESTROYED SITE

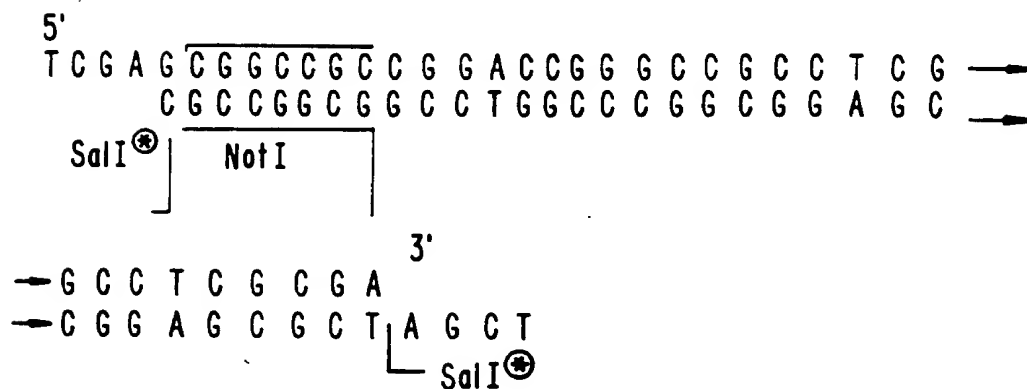


FIG. 23C.

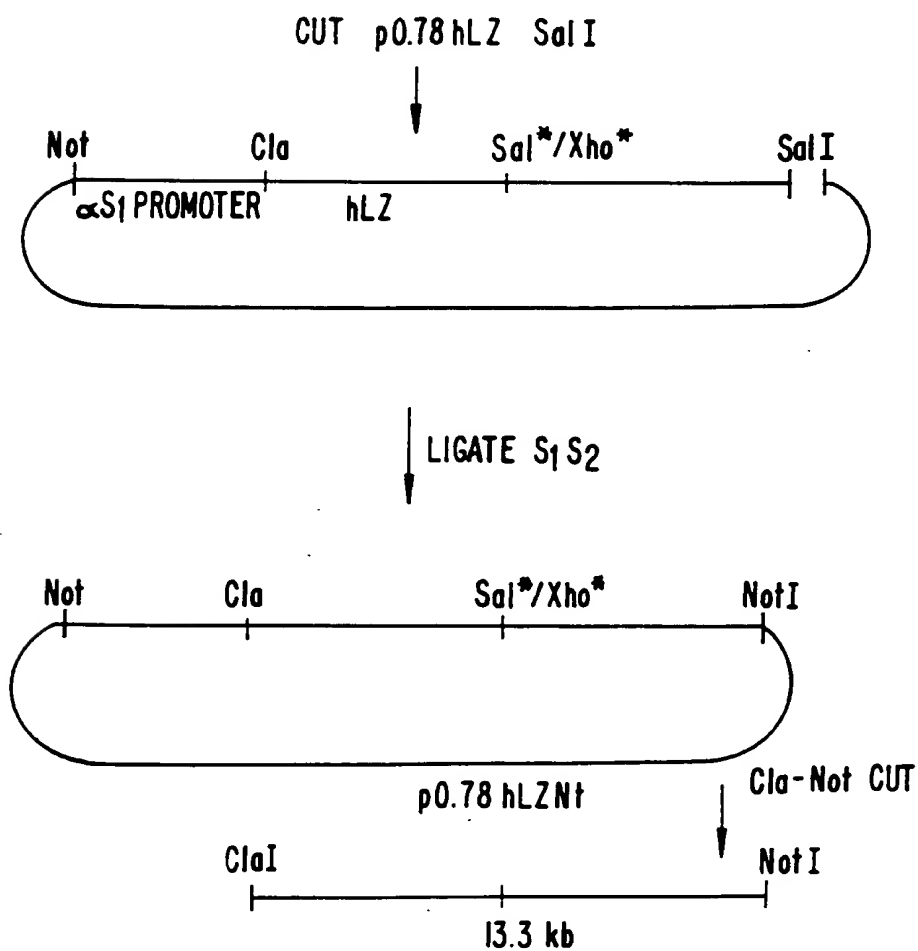
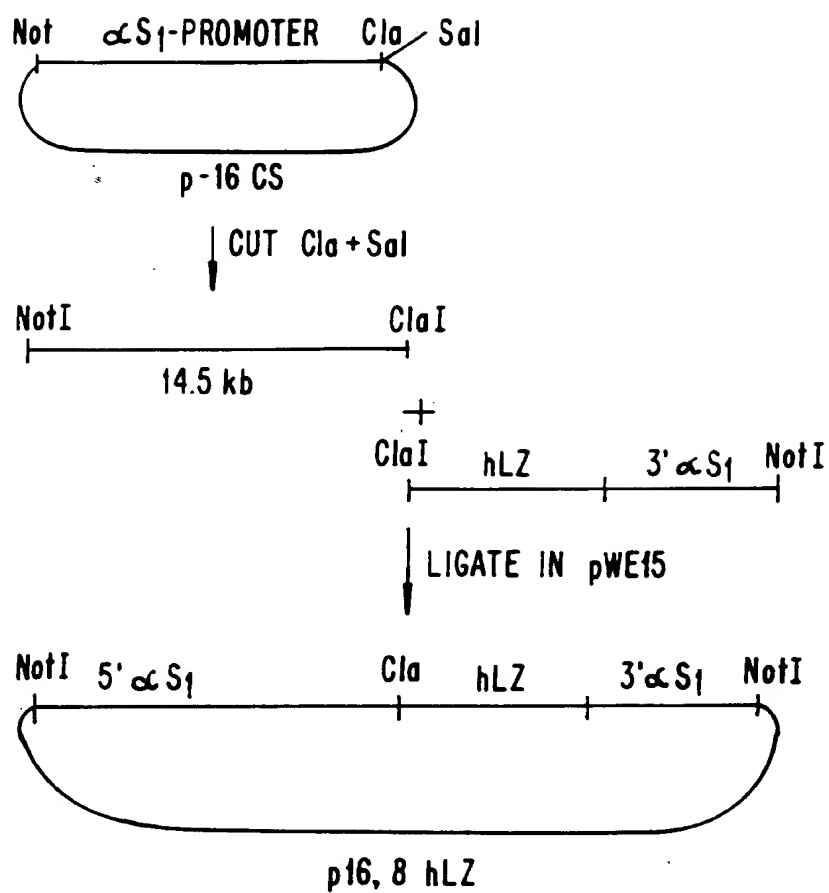


FIG. 23D.

**FIG. 23E.**

BOVINE 2 GGAAGTGCCTGGAGA...TTAAAATGTGAGAGTGGAGT...GGAGGTTG 44
 SHEEP 84 GGAAGTGTCTGCTGGGAGATTATAAATGTGAGAGGCGGGAGGTGGGAGGTTG 133
 45 GGTCTGTAGGCCCTTCCCATCCCACGTGCCTCACGGAGCCCTAGTGCTAC 94
 134 GGGCCTGTGGGCTGCCCATCCCACGTGCCTGCATTAGCCCCAGTGCTGC 183
 95 TCAGTCATGCCCCCGCAGCAGGGGTGAGGTCACTTTCCCATCCTGGGGGT 144
 184 TCAGCCGTGCCCCCGCCGAGGGGTGAGGTCACTTTCCCGTCTT.GGGGT 232
 145 TATTATGACTGTTGTCATTGTTGTTGCCATTTTTGCTACCCTAACTG66C 194
 233 TATTATGACTCTTGTCATTGCCATTGCCATTTTTGCTACCCTAACTG66C 282
 195 AGCGGGTGCTTGCAGAGCCCTCGATACTGACCAGGTTCCCCCTCG6A6C 244
 283 AGCAGGTGCTTGCAGAGCCCTCGATACCGACCAGG.TCCTCCCTCG6A6C 331
 245 TCGACCTGAACCCCATGTCACCCTCGCCCCAGCCTGCAGAGGGTGGGTGA 294
 332 TCGACCTGAACCCCATGTCACCCTTGGCCCCAGCCTGCAGAGGGTGGGTGA 381
 295 CTGCAGAGATCCCTTTACCCAAGGCCACAGTCAATGGTTTGGAGGAGAT 344
 382 CTGCAGAGATCCCTTACCCAAGGCCACGGTCAATGGTTTGGAGGAGCT 431
 345 GGTGCCCAAGGCAGAGCCACCCTCCA.GACACACCTGCCCCCAGTGCTG 393
 432 GGTGCCCAAGGCAGAGGCCACCCTCAGGACACACCTGTCCCCCAGTGCTG 481
 394 GCTCTGACCTGTCTTGTCTAAGAGGCTGACCCAGAAAGTGTTCCTGGCG 443
 482 GCTCTGACCTGTCTTGTCTAAGAGGCTGACCCCGGAAGTGTTCCTGGCA 531
 444 CTGGCAGCCAGCCTGGACCCAGAGCCTGGACACCC.CCTGCGCCCCCACT 492
 532 CTGGCAGCCAGCCTGGACCCAGAGTCCAGACACCCACCTGTGCCCCCGCT 581
 493 TCTGGGGGCGTACCAGGAACCGTCCAGGCCCAGA..GGGCTTCCTGCTT 540
 582 TCTGGGGTC.TACCAGGAACCGTCTAGGCCCAGAGGGGGACTTCCTGCTT 630
 541 GGCTCGAATGGAAGAAAGCCTCCTATTGTCCTTCGTAGAGGAAGCAACC 590
 631 GGCTTGGATGGAAGAAAGCCTCCTATTGTCC.TCGTAGAGGAAGCCACC 679
 591 CGAGGGCCCAAGGATAGGCCAGGGGGATTG66GAACCGCGTGGCT.CC 639
 680 CCGGGGCTGAGGATGAGCCAAGTGGGATTCCGGGAACCGCGTGGCTGGG 729
 640 GGGGGGGGGGGGGCTGGCTGGCTGGC..CCTCCTCCTGTATAAGGCCCG 687
 730 GGCCCAAGCCGGGGCTGGCTGGCTGCATGCGCCTCCTGTATAAGGCCCA 779

FIG. 24

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688 AGCCCG. CTGTCTCAGCCCTCCACTCCCTGCAGAGCTCAGAAGCGTGACC 736
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
780 AGCCTGCCTGTCTCAGCCCTCCACTCCCTGCAGAGCTCAGAAGCACGACC 829
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
737 CCAGCTGCAGCCATGAAGTGCCTCCTGCTTGC.....CCTGGCCCTCAC 780
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
830 CCAGCTGCAGCCATGAAGTGCCTCCTGCTTGGCCCTGGGCCTGGCCCTCGC 879
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
781 CTGTGGCGCCCAAGGCCCTCATCGTCACC 808
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
880 CTGTGGCGTCCAGGCCATCATCGTCACC 907
    ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

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* TRANSLATION INITIATION CODON

FIG. 24 [continued]

LINKER GP 278/279

TRANSLATION START SITE (αS_1 SIGNAL SEQUENCE)
ClaI
 CGATAACCATGAACTTCTTATCCTCACCTGTCTTGTGGCTGTTGCTCTTG
 |
 TATT — ETC.

αS_1 — HLZ SEQUENCE
 — CCAAGGTCTTTGAAAGGTGTGAGTTGC
 ETC. — AACC

FIG. 25.

CONSTRUCTION OF 16.8 A hLZ3:

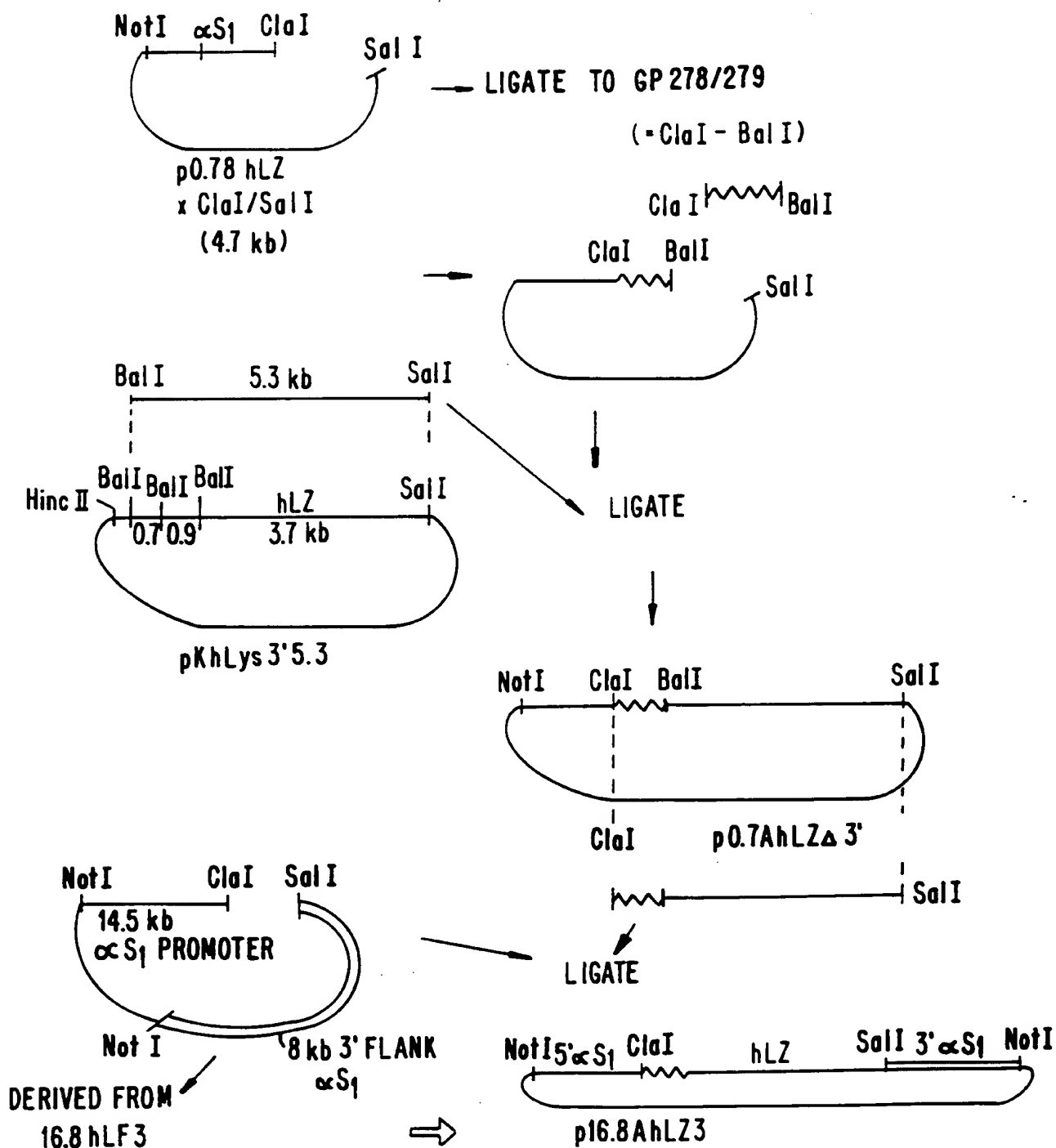


FIG. 26.

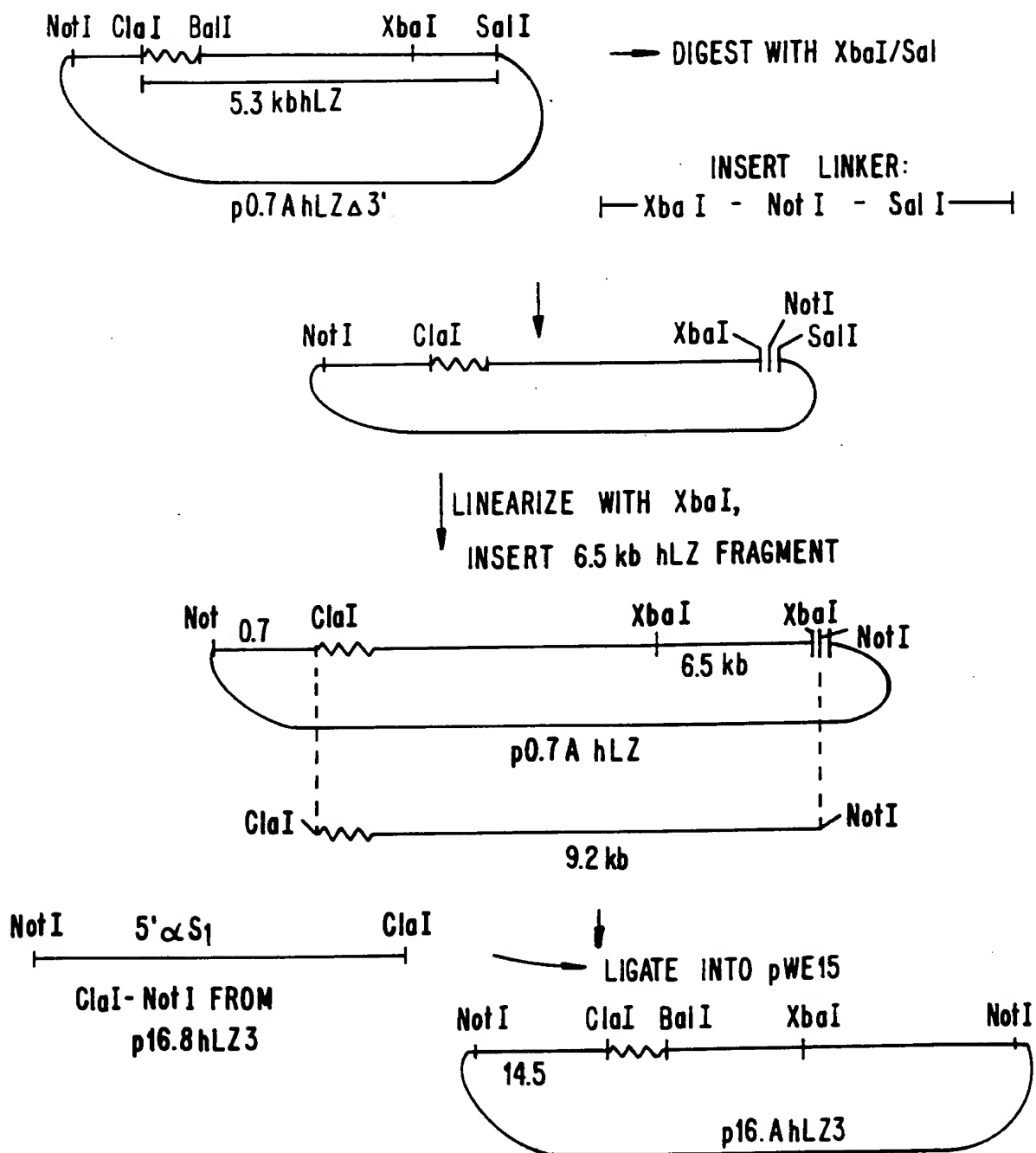
CONSTRUCTION OF 16 A hLZ3:

FIG. 27.